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FROM THE STATE SERUM INSTITUTE, HELSINKI

SERUM CONCENTRATION OF DIFFERENT COMMERCIAL PENICILLIN PREPARATIONS

IV

DIFFERENT BATCHES OF THE SAME PRODUCT

by

VEIKKO TOMMILA and TAPIO SAVOLAINEN

(Received for publication February 17, 1955)

In our earlier studies (1, 2, 3) we found significant differences in the mean penicillin concentrations attained in the serum of hospital patients during 24 hours following intramuscular injection of different penicillin preparations.

In the present study it was our purpose to compare the serum penicillin concentrations obtained with different batches of some penicillin preparations.

The penicillin concentration determinations made from the serum after a single intramuscular injection of 600.000 units into the gluteal region, and the determinations of the unit content of the products were carried out by the techniques described in the previous reports (1, 2, 3, 4, 5).

The test subjects were 80 adult patients hospitalised in the Helsinki University Hospital for Ear, Nose and Throat Diseases and the First Central Military Hospital.[1] The age of the patients ranged from 17 to 66 years and their body weights from 48 to 80 kg.

[1] We gratefully acknowledge the kindness of Professor Y. Meurman, M. D., Chief of the Helsinki University Hospital for Ear, Nose and Throat Diseases, and Major-General P. Somer, M. D., Chief Medical Officer of the Finnish Army, in placing at our disposal a sufficiently large series of patients.

RESULTS

The results are made clear by the appended graphs (Figs. 1—8), which show the mean serum concentrations obtained during 24 hours with the different batches. The products are designated by capital letters, the small figures indicate the numbers of the preparations of each manufacturer, and small letters the different batches. The mean maximum and 24-hour concentrations in units per ml of serum, with their standard deviations in parentheses[1], and the number of patients tested are also listed.

Aqueous Suspensions with Procaine Penicillin G. — There were a total of 11 batches of five different preparations which contained only procaine penicillin G in aqueous suspension. The results are shown in Figs. 1, 2 and 3.

Our determinations of the unit penicillin content of the products stated in the figures gave the following results: different batches of product C_2 (11 batches), 290.000—360.000 units per ml of preparation; product D_1, 300.000—310.000 units per ml; product F_1, 350.000—380.000 units per ml; and product B_1, batch »a», 470.000 units per ml. According to manufacturer's determinations batch »b» contained 350.000 units per ml.

From the above graphs (Figs. 1, 2 and 3) it is observed that the three different baches of product C[2] gave very similar concentration curves, which showed no marked differences in height, *i.e.*, in the pen.cillin concentration in the serum. In consideration of the

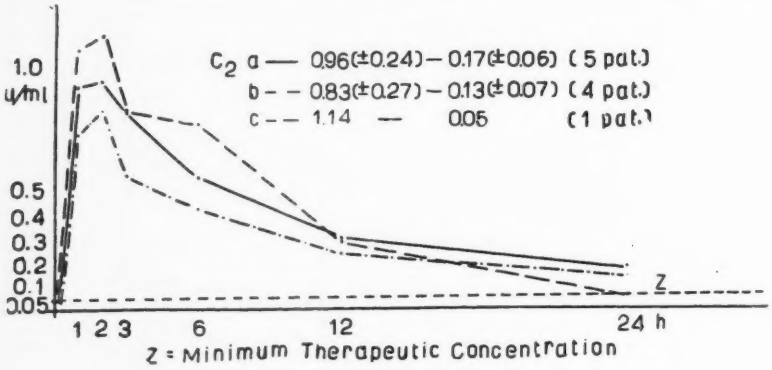

Fig. 1.

[1] We are indebted to Mr. E. Kaila, Ph. D., for assistance in controlling the mean values and calculating the standard deviations.

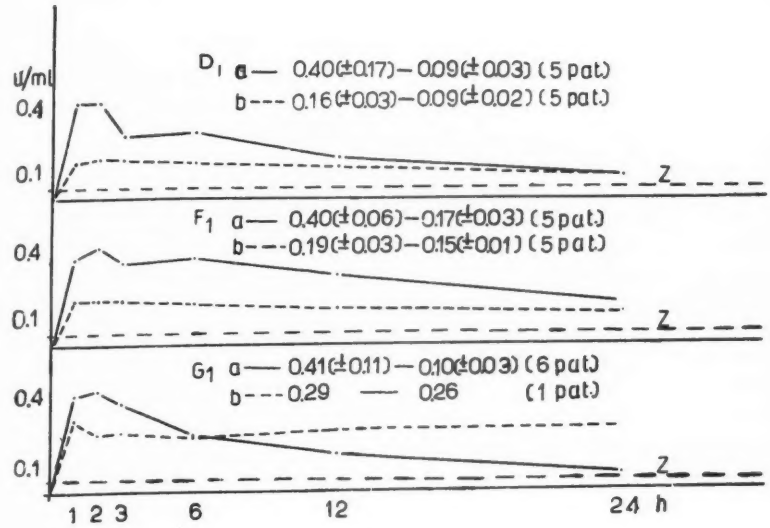

Fig. 2.

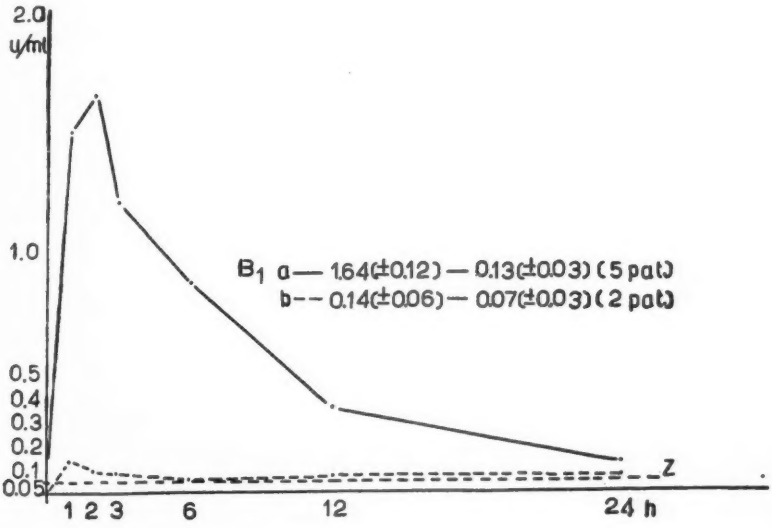

Fig. 3.

Figs. 1, 2 and 3. — Average penicillin concentrations and their standard deviations in sera of adult hospital patients during 24 hours after a single intramuscular injection of 600,000 units of different batches of procaine penicillin G preparations in aqueous suspension.

standard deviations of the mean values, it is concluded that no dissimilarity exists between the examined batches of product C_2.

Products D_1 and F_1, of which two batches each were tested, gave concentration curves of nearly the same type. The batches differed in the early part of the curves and especially in the maximum concentrations, whereas the concentrations at 24 hours were the same or nearly so. When the standard deviations of the mean and the variations unavoidably occurring in the unit content of the different batches are taken into consideration, the different batches of these two products cannot be regarded as dissimilar in essential respects. One of the batches of product G_1 was tested on one patient only and no definite conclusions can therefore be drawn. However, there is nothing to indicate even in this case that the different batches would not be similar.

The two batches tested of product B_1 gave greatly diverging results in both the form and the height of the concentration curves. At maximum concentration the differences were nearly 12-fold (1.64 u./ml serum as against 0.14 u./ml serum). Batch »a», it is true, which reached the higher concentrations had a considerably higher unit content of penicillin (470.000 u./ml) than batch »b» or any other product tested. In view of also the standard deviations, the two batches of product B_1 which were examined cannot be regarded as similar but rather as essentially different.

All the above mentioned products and their different batches were able to maintain in the serum of hospital patients during 24 hours a penicillin concentration which exceeded the minimum therapeutic concentration.

Oily Suspensions with Procaine Penicillin G. — There were two preparations, J and G_2, which contained only procaine penicillin G in oily suspension. Two different batches of product J were tested. Of the product G_2 there was tested only one batch, but the test was repeated after six months' storage in the icebox. The results are given in Figs. 4 and 5.

The unit content determinations made by us showed that product J contained 300.000—340.000 u./ml and product G_2 340.000 u./ml.

Curves of a similar type were obtained for the average serum concentrations of the two batches of product J; however, batch »a» gave higher values than batch »b». In view of the results ob-

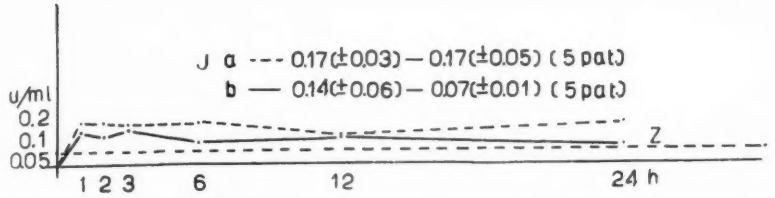

Fig. 4. — Average penicillin concentrations and their standard deviations in sera of adult hospital patients during 24 hours after a single intramuscular injection of 600,000 units of two different batches of a commercial penicillin G product in oity suspension.

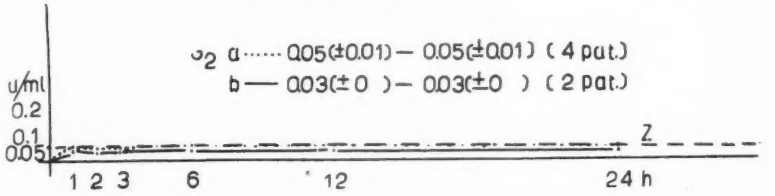

Fig. 5. — Average penicillin concentrations and their standard deviations in sera of adult hospital patients during 24 hours after single intramuscular injections of 600,000 units of a commercial penicillin G product in oily suspension at an interval of six months.

tained and the standard deviations of the mean values, there is no reason to consider the two batches different. Both batches, furthermore, maintained in the serum of hospital patients during 24 hours a penicillin concentration exceeding the minimum therapeutic concentration.

The tests with product G_2 differed from the foregoing tests in being carried out with the same batch at two different times. They were first made with two patients, and six months later on four patients, the preparation being stored in the meantime in the refrigerator at +4°C. In both cases the results were of the same type, but the concentrations obtained after storage for six months in the refrigerator were slightly higher. The differences, however, were relatively small and therefore, taking into consideration the standard deviations of the mean, they presumably have little significance. The penicillin concentrations in the serum were in most cases below the therapeutic minimum or were slightly above this concentration temporarily only.

Aqueous Suspensions with Procaine and Sodium Penicillin G. — There were a total of 8 different batches of four different preparations which contained procaine penicillin G, 75 per cent, and

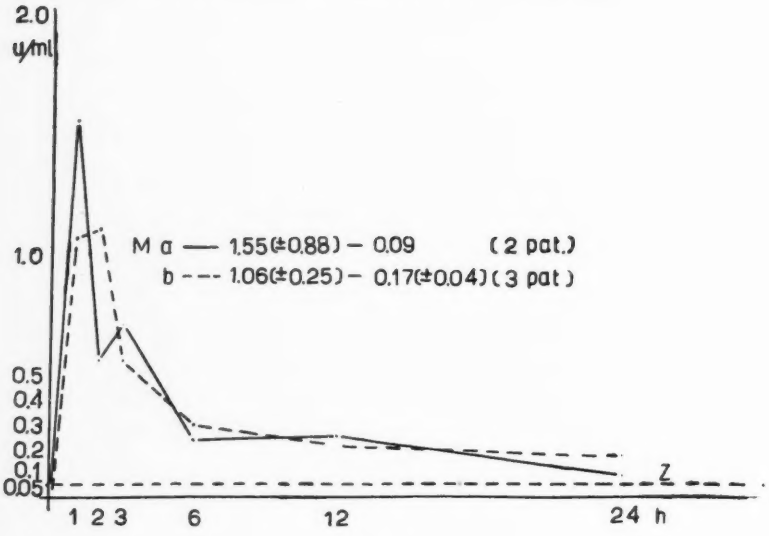

Fig. 6.

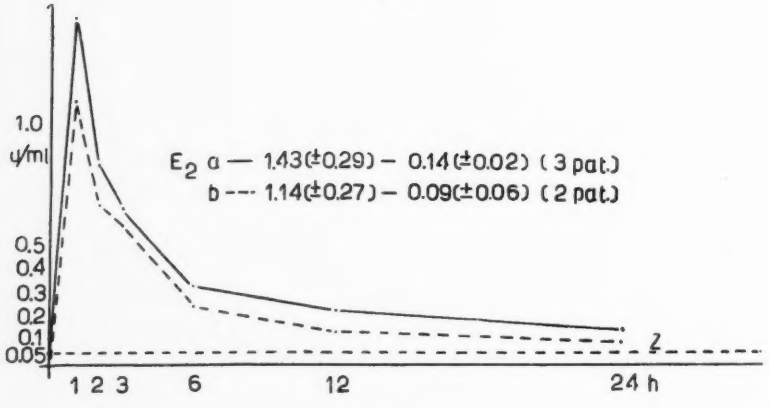

Fig. 7.

Figs. 6, 7, 8 and 9. — Average penicillin concentrations and their standard deviations in sera of adult hospital patients during 24 hours after a single intramuscular injection of 600,000 units of different batches of commercial penicillin preparations containing 75 % procaine and 25 % sodium penicillin G in aqueous suspension.

sodium penicillin G, 25 per cent, in aqueous suspensions. The results are shown in Figs. 6, 7, 8 and 9.

The unit content determinations made by us showed that product C_3 contained 300.000—400.000 u./ml of product.

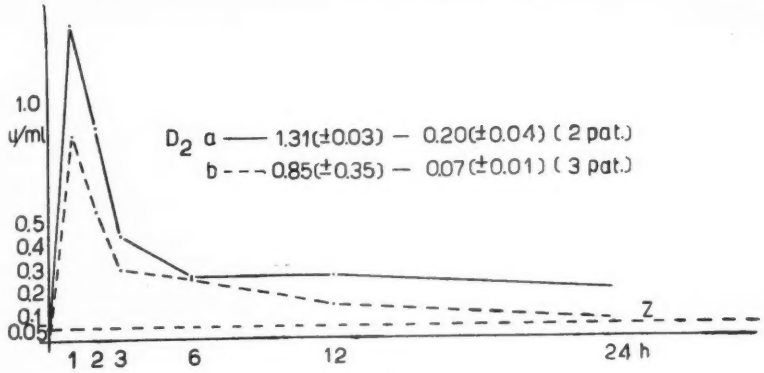

Fig. 8.

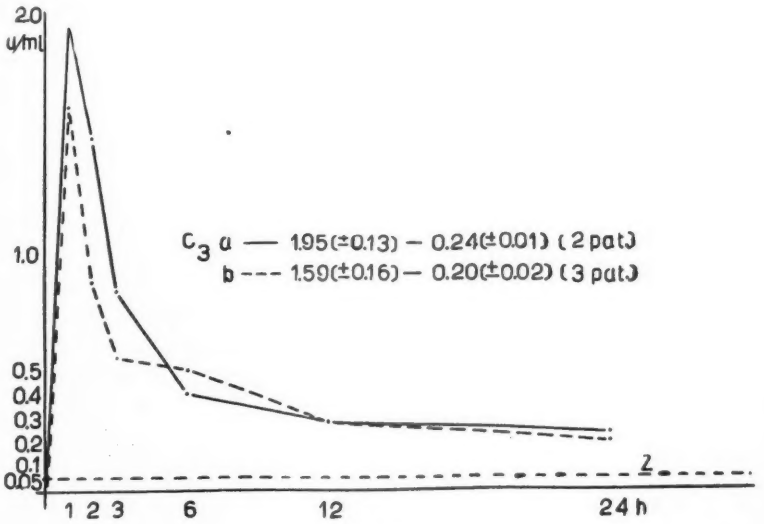

Fig. 9.

As is shown in Figs. 6, 7, 8 and 9, we tested two batches of each of four products containing both procaine and sodium penicillin G (products M, E_2, D_2 and C_3). The curves obtained were of a very similar type as to both shape and level. With consideration also to the standard deviations there appears to be no reason to consider the different batches dissimilar.

All these products and their different batches gave a penicillin concentration in the serum of hospital patients that exceeded the minimum therapeutic concentration during 24 hours.

SUMMARY

1. Using the technique described in earlier papers for determination of the penicillin concentration in the serum, 22 batches of 11 different commercial penicillin products were tested on 80 adult hospital patients. The products tested were five preparations of procaine penicillin G in aqueous suspension, one preparation of procaine penicillin G in oily suspension and four preparations containing procaine and sodium penicillin G in aqueous suspension. A further test was made using the same batch of procaine penicillin G in oily suspension at an interval of six months.

2. The two batches of one procaine penicillin G product in aqueous suspension differed essentially, the average maximum concentrations showing a nearly 12-fold difference (0.14 as against 1.64 u./ml serum). The different batches of other products gave similar or nearly similar results. The results obtained at an interval of six months with the same batch of procaine penicillin G in oily suspension yielded approximately similar results.

3. All the products with the exception of one procaine penicillin G preparation in oily suspension maintained, when administered to hospital patients in a single intramuscular dose of 600,000 units, during 24 hours an average penicillin concentration in the serum which exceeded the minimum therapeutic concentration.

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FROM THE STATE SERUM INSTITUTE, HELSINKI

SERUM CONCENTRATION OF DIFFERENT COMMERCIAL PENICILLIN PREPARATIONS

V

EFFECT OF MOVEMENT ON PENICILLIN LEVEL

by

TAPIO SAVOLAINEN and VEIKKO TOMMILA

(Received for publication July 18, 1955)

In their tests with one preparation containing procaine penicillin G in oily suspension, Boger *et al.* (1) observed differences in the serum penicillin levels of hospital and out-patients. Thus with a dose of 300,000 units they were able to maintain a serum penicillin concentration of over 0.039 u./ml of serum for an average of 12 hours (6—30 hours) in afebrile and ambulatory patients (9 patients) and for an average of 33 hours (7—70 hours) in patients with pyrexia and pneumonia (11 patients). In both groups the maximum concentration was 0.34—2.7 u./ml of serum.

PRESENT INVESTIGATION

Since our previous unpublished investigations seemed to indicate that certain penicillin preparations gave higher serum penicillin concentrations in out-patients than in hospital patients, we have extended our study to include tests with certain penicillin preparations on out-patients or healthy and working subjects in one group and hospital patients in another group. Observations were made of the mean serum penicillin concentration obtained in these groups during 24 hours following a single intramuscular injection of 600,000 units into the gluteal region.

The series of hospital patients, manner of procurement of the penicillin preparations, and the methods of determination of the penicillin content per unit and of the serum penicillin concentration were the same as in our previous tests (2, 3, 4). The ambulatory series consisted partly of out-patients from the Municipal Policlinic for Venereal Diseases [1] and partly of healthy persons working in the State Serum Institute.

RESULTS

The results obtained with the various types of penicillin preparations are depicted graphically in Figs. 1—8.

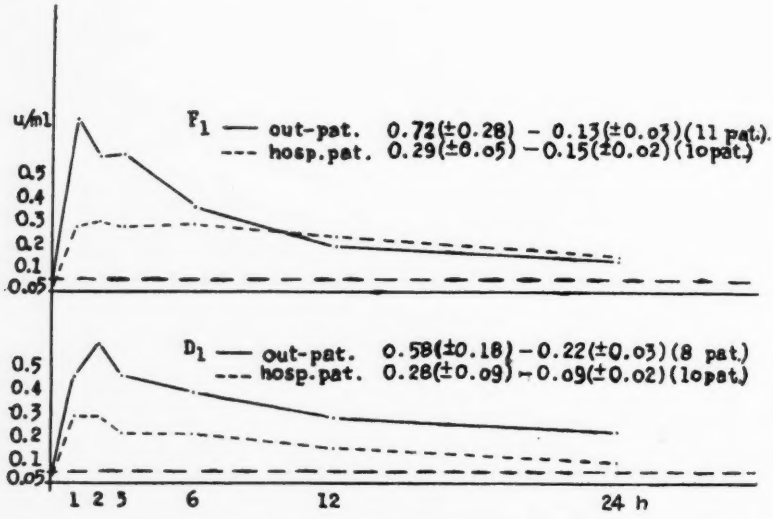

Fig. 1. — Mean penicillin concentrations in sera of hospital patients and of out-patients or healthy working subjects during 24 hours after a single intramuscular injection of 600,000 units of commercial preparations containing procaine penicillin G in aqueous suspension (product F_1: penicillin content 350,000—360,000 u./ml of preparation; product D_1: 300,000—360,000 u./ml of preparation) [2] [3].

[1] We gratefully acknowledge the kindness of Y. Salminen, M.D., medical officer of the City of Helsinki, in placing at our disposal a number of patients in the Municipal Policlinic for Venereal Diseases.

[2] The preparations are designated by letters; small figures indicate the numbers of the products of each manufacturer. The following figures are the mean maximum concentration (in units per ml of serum), its standard deviation, mean concentration 24 hours after injection, its standard deviation, and number of test subjects.

[3] We gratefully acknowledge the assistance of Mr. E. Kaila, Ph.D., in checking the mean values and calculating the standard deviations.

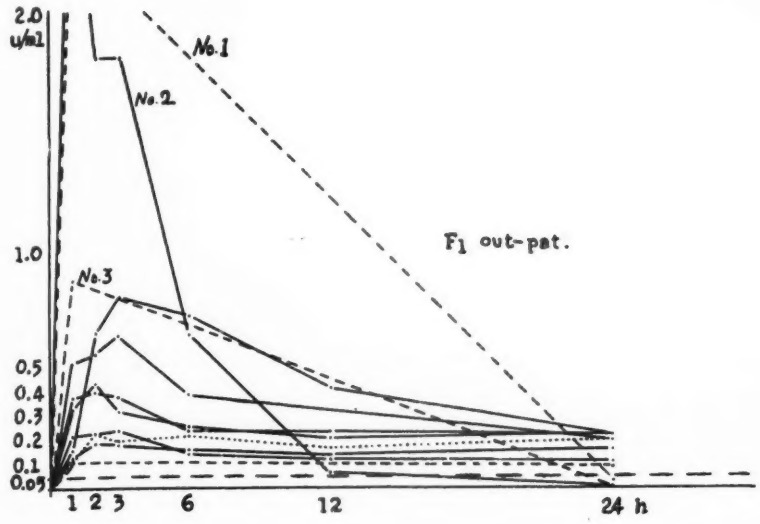

Fig. 2.

Figs. 2, 3, 4, 5. — Individual values for each patient in the groups shown in Fig. 1.

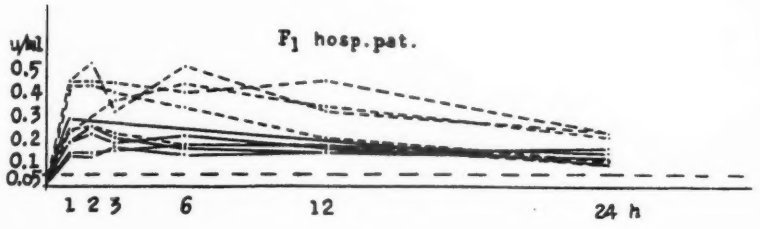

Fig. 3.

Preparations Containing Procaine Penicillin G in Aqueous Suspension. — Figs. 1—5 show the results for two procaine penicillin G preparations in aqueous suspension.

It will be observed from Fig. 1 that products F_1 and D_1 gave different mean values for hospital patients and for out-patients or healthy working subjects. A higher mean maximum concentration was obtained with both products in ambulatory persons than in hospital patients ($F_1 = 0.72$ as against 0.29 u./ml of serum; $D_1 = 0.58$ as against 0.28 u./ml of serum). Product D_1 also gave a higher mean concentration at 24 hours for the ambulatory persons (0.22 as against 0.09 u./ml of serum), whereas with product F_1 the serum

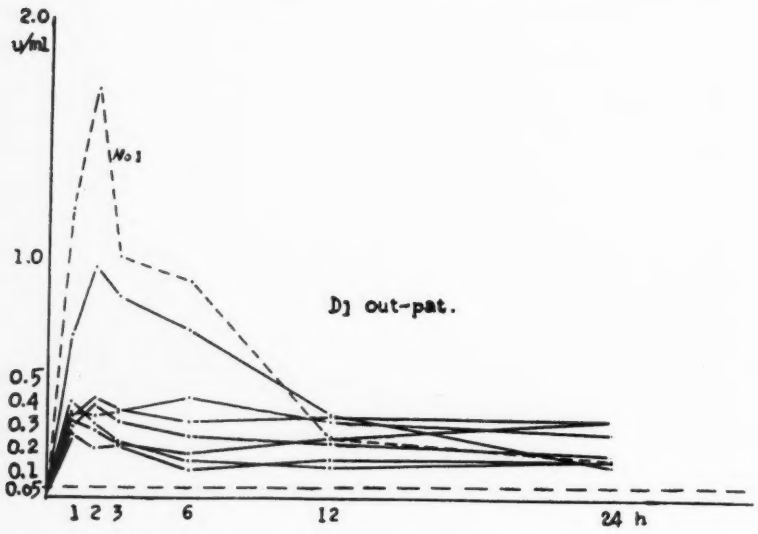

Fig. 4.

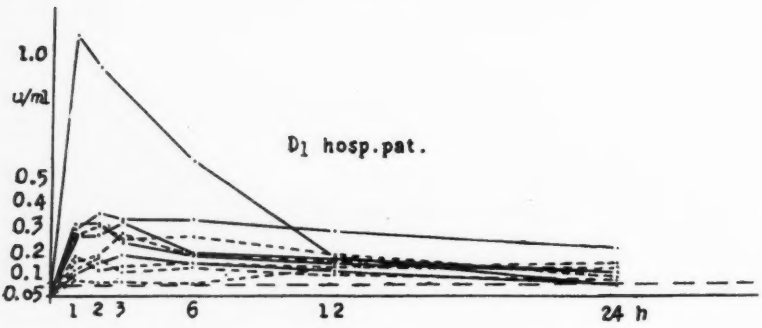

Fig. 5.

penicillin concentration was higher in the ambulatory persons during 10 hours only, the values at 24 hours being 0.13 u./ml of serum for ambulatory persons and 0.15 u./ml of serum for hospital patients.

As will be seen from the individual curves for all the patients (Figs. 2, 3, 4 and 5), certain ambulatory subjects showed markedly high serum penicillin concentrations (Fig. 4: product D_1 = ad 1.70 u./ml of serum; Fig. 2: product F_1 = ad 2.80 u./ml of serum). A closer examination of this circumstance seemed to indicate that higher values are obtained for those ambulatory persons who move

about, walk or run much, or at least more than the other test subjects. Such persons were subject No. 1 in Fig. 4 and No. 1 in Fig. 2. It may be probable that the muscular movement in walking and running promote the resorption of penicillin from the gluteal region. On the other hand, it was unexpected that certain subjects had high serum penicillin concentrations after exertion. Thus the maximum concentrations of 2.80 u./ml of serum in Fig. 2, curve 2 and of 0.87 u./ml of serum in curve 3 were seen on the morning following several rounds of, respectively, basket ball and tennis on the previous evening. That these actually were cases in which motion has an influence on the resorption was proved by a repeat test made under similar conditions except for the basket ball game on the preceding evening. An identical dose of the same batch of penicillin then gave a maximum concentration of 0.30 u./ml of serum.

Preparation Containing Procaine Penicillin G in Oily Suspension. — The results for this preparation are shown in Fig. 6.

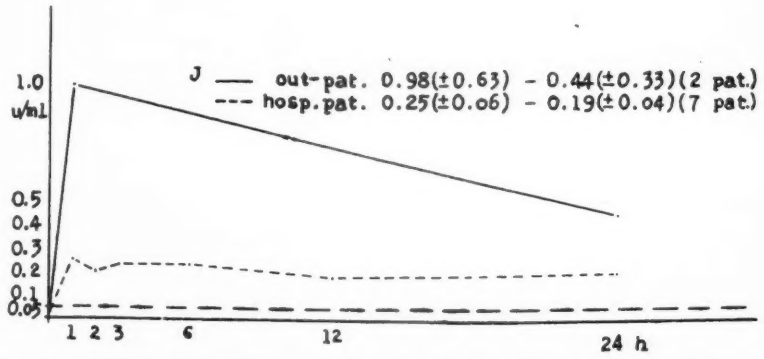

Fig. 6. — Mean penicillin concentrations in sera of hospital patients and out-patients during 24 hours after a single intramuscular injection of preparation J containing procaine penicillin G in oily suspension (penicillin content 300,000—340,000 u./ml of preparation).

It will be observed that product J also gave a considerably higher mean maximum concentration in the ambulatory group than in the hospital group (0.98 as against 0.25 u./ml of serum). The same was true of the concentration at 24 hours (0.44 as against 0.19 u./ml of serum). However, since only two ambulatory patients were tested, the mean value cannot be considered fully convincing, but a noteworthy finding is the high maximum concentration (1.61 u./ml of

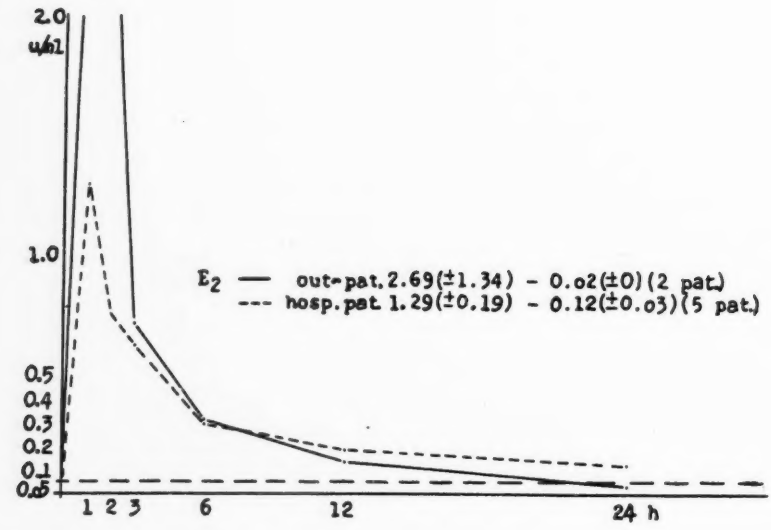

Fig. 7.

Figs. 7 and 8. — Mean penicillin concentrations in sera of hospital patients and out-patients during 24 hours after a single intramuscular injection of 600,000 units of commercial preparations E_2 and D_2 containing 75 per cent of procaine and 25 per cent of sodium penicillin G in aqueous suspension.

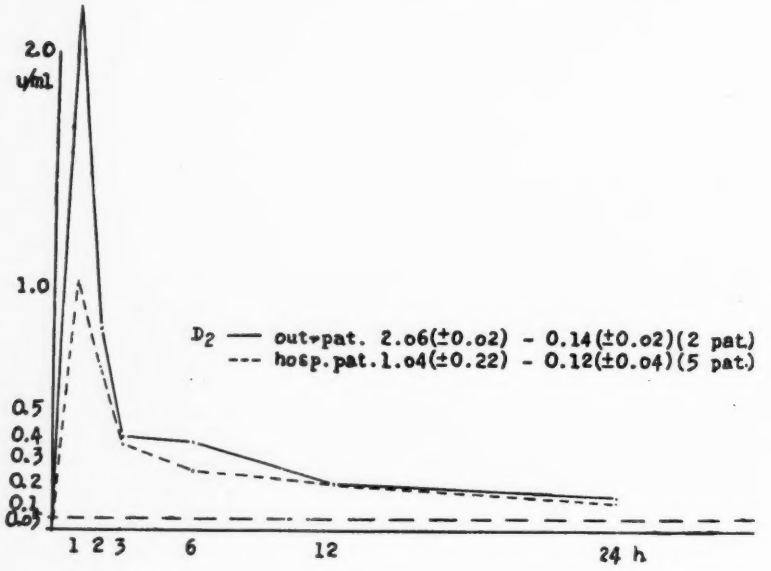

Fig. 8.

serum) and likewise high 24-hour concentration (0.77 u./ml of serum) seen in one of the two patients, who performed heavy labour.

Preparations Containing 75 Per Cent Procaine Penicillin G and 25 Per Cent Sodium Penicillin G in Aqueous Suspension. — The results obtained with two products of this type are shown in Figs. 7 and 8.

As will be seen, the aqueous suspensions E_2 and D_2 containing procaine and sodium penicillin gave definitely higher maximum concentrations in the ambulatory subjects than in hospital patients (E_2 = 2.69 as against 1.29 u./ml of serum; D_2 = 2.06 as against 1.04 u./ml of serum). Twenty-four hours after injection the serum penicillin concentration in the out-patients receiving E_2 was lower (0.02 as against 0.12 u./ml of serum) and in those receiving D_2 higher (0.14 as against 0.12 u./ml of serum) than in the hospital patients.

As is only natural after a single injection of penicillin, it appears that the higher the maximum concentration in ambulatory persons reaches, the more abruptly and rapidly it declines.

SUMMARY

1. Tests were made to determine the effect of movement on the serum penicillin concentration after a single intramuscular injection of 600,000 units of penicillin into the gluteal region, using the *S. lutea* method to determine the penicillin level in the serum of hospital patients and the serum of out-patients or healthy working persons. The preparations tested were two procaine penicillin G preparations in aqueous suspension, one procaine penicillin G preparation in oily suspension, and two preparations containing procaine and sodium penicillin G in aqueous suspension.

2. Two preparations of procaine penicillin G in aqueous suspension gave more than twice as high mean maximum serum penicillin concentrations in ambulatory subjects than in hospital patients (0.72 as against 0.29 u./ml of serum; 0.58 as against 0.28 u./ml of serum). The difference in the values at 24 hours was over 2-fold (0.22 as against 0.09 u./ml of serum) in the case of one of the products. With the other product the serum penicillin concentration for the ambulatory subjects remained for 10 hour higher than

for the hospital patients; at 24 hours the concentrations were approximately equal (0.13 as against 0.15 u./ml of serum).

3. With the preparation in oily suspension, considerably higher maximum and 24-hour serum penicillin concentrations were obtained for out-patients than for hospital patients (maximum: 0.98 as against 0.25 u./ml of serum; at 24 hours: 0.44 as against 0.19 u./ml of serum).

4. It was also observed that the individual serum penicillin values increased in proportion to the amount of movement after injection of the aqueous or oily suspension of procaine penicillin G, and that significantly high values were obtained when the injection was given after strenuous physical exertion.

5. With two preparations containing both procaine and sodium penicillin G in aqueous suspension, twice as high maximum serum penicillin concentrations were obtained for ambulatory persons than for hospital patients (with one product 2.69 as against 1.29 u./ml of serum; with the other product 2.06 as against 1.04 u./ml of serum). The serum penicillin level remained higher for 7—24 hours in ambulatory subjects than in hospital patients.

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FROM THE STATE SERUM INSTITUTE, HELSINKI

SERUM CONCENTRATION OF DIFFERENT COMMERCIAL PENICILLIN PREPARATIONS

VI

EFFECT OF DOSAGE, DURATION OF PENICILLIN CONCENTRATION DURING WORK, COMPARATIVE TESTS WITH HOSPITAL PATIENTS AND WITH BOGER'S EXCHANGE SYSTEM

by

VEIKKO TOMMILA and TAPIO SAVOLAINEN

(Received for publication August 25, 1955)

In this series of tests we studied a) the mean serum penicillin concentrations obtained in hospital patients with varying doses of the same preparation, b) concentrations attainable in out-patients during 3—21 days with certain preparations, and c) comparison of serum penicillin concentrations obtainable with the same penicillin preparation in hospital patients and with Boger's exchange system in out-patients.

The series of hospital patients, manner of procurement of the penicillin preparations, and the method of determination of the penicillin level was the same as in our previous tests (10, 11, 12, 13, 14, 15). The out-patients were partly from the Municipal Policlinic for Venereal Diseases[1] and partly from the State Serum Institute.

RESULTS

The results obtained are shown graphically by groups in Figs. 1—6.

[1] We gratefully acknowledge the kindness of Y. Salminen, M.D., medical officer of the City of Helsinki, in placing at our disposal a number of patients in the Municipal Policlinic for Venereal Diseases.

a) *Effect of Dosage on the Serum Penicillin Level.* — The preparation administered was procaine penicillin G in aqueous suspension, product F_1, of a batch containing 350,000 u./ml according to a previous assay made by us. Doses of 300,000, 600,000 and 1,200,000 u. (according to declaration) were given as a single intramuscular injection into the gluteal region of five hospital patients each, aged 24—66 years and weighing 54—80 kg. The mean serum penicillin concentrations for each group during 24 hours and the standard deviations [1] at maximum concentrations and at 24 hours are shown in Fig. 1.

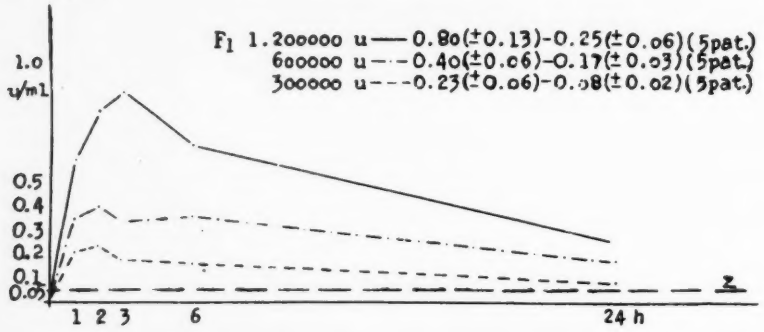

Fig. 1.[2] — Mean penicillin concentrations in sera of five adult hospital patients after a single intramuscular injection of 300,000, 600,000 or 1,200,000 units of preparation F_1 containing procaine penicillin G in aqueous suspension.

As had been observed in an earlier study (7), the serum penicillin levels rise with increasing dosages. In this case the mean rise was almost directly proportionate to the increase in dosage, the mean concentrations obtained for adult hospital patients with product F_1 (procaine penicillin G in aqueous suspension) in the doses used (300,000, 600,000 and 1,200,000 u.) being at maximum 0.23, 0.40 and 0.80 u./ml of serum, respectively, and at 24 hours 0.08, 0.17 and 0.25 u./ml of serum, respectively.

b) *Maintenance of the Serum Penicillin Concentration during Work.* — This test was carried out in two parts, the first dealing with 1) serum penicillin levels attained with procaine penicillin G

[1] We gratefully acknowledge the assistance of Mr. E. Kaila, Ph.D., in controlling the mean values and calculating the standard deviations.

[2] Figures given for each curve in Fig. 1 indicate size of dose, concentrations at maximum and at 24 hours in u./ml of serum, their standard deviations, and the number of test subjects.

and their duration during three days, and 2) levels attained with dibenzylethylenediamine dipenicillin G and their duration during 21 days.

1. *Serum Penicillin Levels in Working Patients during Three Days after Injection of Procaine Penicillin G.* — Since the Helsinki Municipal Policlinic for Venereal Diseases had obtained excellent results with injections at 48-hour intervals of procaine penicillin G in oily suspension, product J, but considerably poorer results with procaine penicillin G in aqueous suspension, product Z, we made random determinations of the serum penicillin concentrations during 72 hours for some of these out-patients occupied in manual labour, in order to determine if a clear correlation exists between the serum penicillin concentrations and the therapeutic results. The results are shown in Fig. 2.

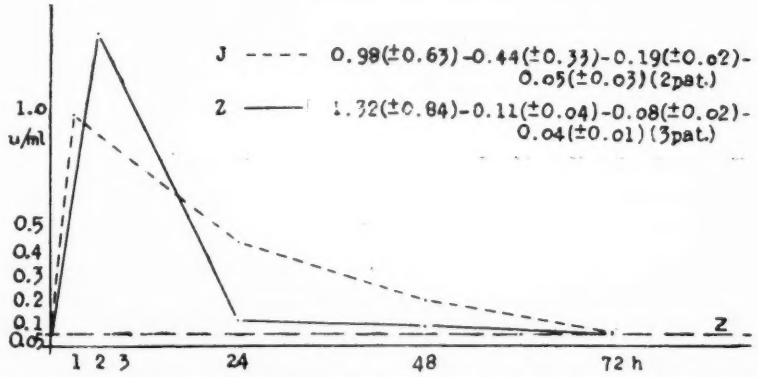

Fig.[1] 2. — Mean penicillin concentrations during 72 hours and their standard deviations in the sera of adult out-patients in manual labour after a single intramuscular injection in the gluteal region of 600,000 units of preparation J containing procaine penicillin G in oily suspension (two patients) and of preparation Z containing procaine penicillin G in aqueous suspension (three patients).

It is noted that product J gave during 72 hours a mean serum penicillin concentration for out-patients which exceeded the minimum therapeutic concentration of 0.05 u./ml of serum. The mean maximum concentration was 0.98 u./ml of serum and the concentrations at 24 and 48 hours 0.44 and 0.19 u./ml of serum, respectively. Since we had earlier produced with the same product in the same dose given to hospital patients (13) mean maximum and 24-hour

[1] Figures in the graph indicate the mean penicillin concentrations at maximum, 24, 48 and 72 hours in u./ml of serum, and their standard deviations.

concentrations of 0.25 and 0.19 u./ml of serum, this is evidence that out-patients in manual work have a nearly four times as high mean maximum concentration as hospital patients, and that the concentration in out-patients was at 48 hours as high as in hospital patients at 24 hours, being 0.19 u./ml of serum. The effect of manual labour in increasing the serum penicillin level is obvious in this case.

Product Z gave a higher mean maximum concentration (1.32 u./ml of serum) than product J (0.98 u./ml of serum), but the concentration curve for the first mentioned product declined after 13—14 hours below that of product J. At 24 hours it was only 0.11 u./ml of serum, *i.e.*, one-fourth of the concentration of product J. The serum penicillin concentrations attained and their maintenance were in our opinion in agreement with the therapeutic results obtained with the tested products.

2. *Serum Penicillin Levels in Working Patients during 21 Days after Injection of Dibenzylethylenediamine Dipenicillin G.* — Since introduction of N,N'-dibenzylethylenediamine dipenicillin G on the market it has been found to maintain a demonstrable serum penicillin level for a very long time, *i.e.*, for 20 days or longer (1, 6, 8, 9, 16). Using a dose of 600,000 units either intramuscularly or per os, mean maximum concentrations of 0.1—0.2 u./ml of serum, or slightly more were obtained (1, 6, 9, 16).

Having been able to secure N,N'-dibenzylethylenediamine dipenicillin G in aqueous suspension and having observed the increase in the serum penicillin level produced by movement (13), we administered this product in a single intramuscular dose of 2.4 million units to two out-patients with recent syphilis (married couple). The serum penicillin determinations were carried out during 21 days. The individual results are shown in Fig. 3.

As is seen from this figure, the product maintained during 21

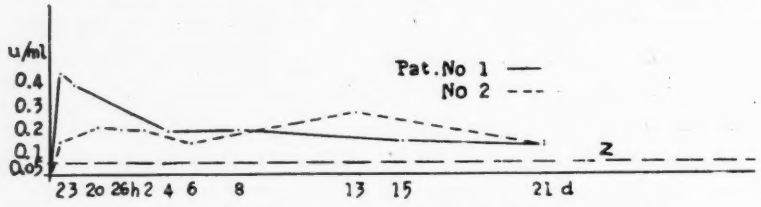

Fig. 3. — Individual penicillin concentrations during 21 days in the sera of out-patients occupied in manual work, after a single intramuscular injection of 2.4 million units of N,N'-dibenzylethylenediamine dipenicillin G in aqueous suspension.

days a serum penicillin concentration exceeding 0.12 u./ml of serum in the tested patients engaged in manual work. The maximum values were 0.43—0.25 u./ml of serum. It deserves to be noted that the maximum concentration for one of the patients was not reached before the thirteenth day.

c) *Comparative Tests with Hospital Patients and with Boger's Exchange System.* — In carrying out their determinations of serum penicillin concentrations, Boger (2), Boger *et al.* (3, 4, 5, 6) and Bayne *et al.* (1) have sought to eliminate possible individual differences in the test subjects which may affect the results, by different penicillin preparations on the same persons under the same conditions.

To obtain a clear picture of the reliability of the results obtained with our own series of patients we carried out a comparison with the same product between the results obtained for hospital patients and those obtained with Boger's method (2) for out-patients. For this purpose, products F_1 and D_1 containing procaine penicillin G in aqueous suspension were given as a single intramuscular injection into the gluteal region of five hospital patients each, *i.e.*, to a total of ten patients aged 26—66 years and weighing 56—73 kg. Similar doses from the same batches of these products were given to six healthy persons engaged in light work in the State Serum Institute, administering both products to each person. The latter group comprised five female and one male subject aged 24—41 years and weighing 54—82 kg. Simultaneous injections were given according to Boger of product F_1 to three subjects and of product D_1 to the three other subjects. The test was repeated in the reverse order on the same day a week later under the same conditions, those previously given product F_1 now receiving product D_1, and *vice versa*. Figs. 4, 5 and 6 show the individual and mean serum penicillin concentrations for the hospital patients and for the persons in light work.

The serum of some of the latter subjects still showed a weak bacteriostatic action one week after injection, as seen in Figs. 5 and 6, in which the concentration curves for these persons starts above 0 point.

It was necessary to repeat the test for some of the working subjects. Two of these developed an irritation of the skin, and had to be substituted by other persons. The results obtained for the

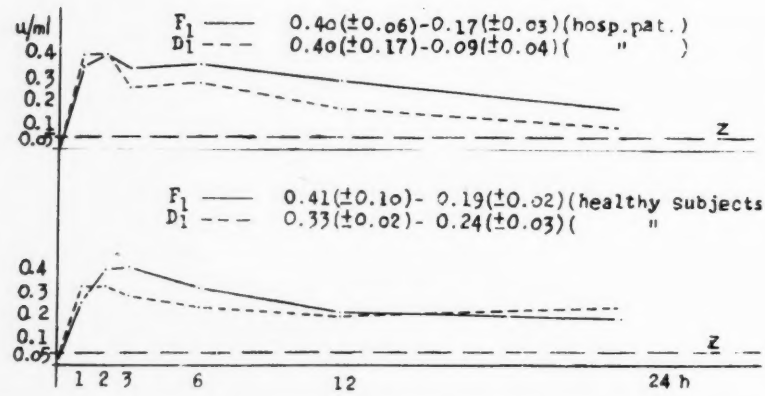

Fig. 4.[1] — Mean penicillin concentrations during 24 hours and their standard deviations in the sera of five adult hospital patients (two separate series) and six healthy adults in light work (same series) after a single intramuscular injection of 600,000 units of products F_1 and D_1 containing procaine penicillin G in aqueous suspension.

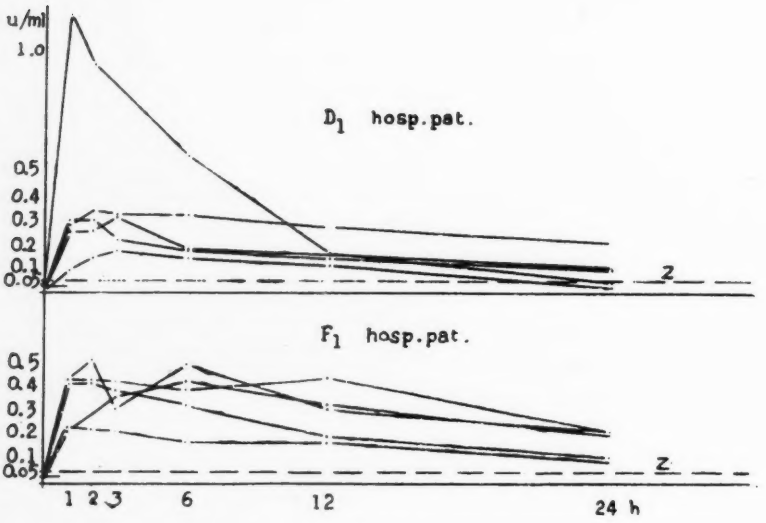

Fig. 5.

[1] Figures in the graph indicate the mean penicillin concentrations at maximum and at 24 hours in u./ml of serum, and their standard deviations. Unit content of product F_1: 350,000 u./ml of product; D_1: 300,000 u./ml of product. Size of dose was calculated according to manufacturer's declaration.

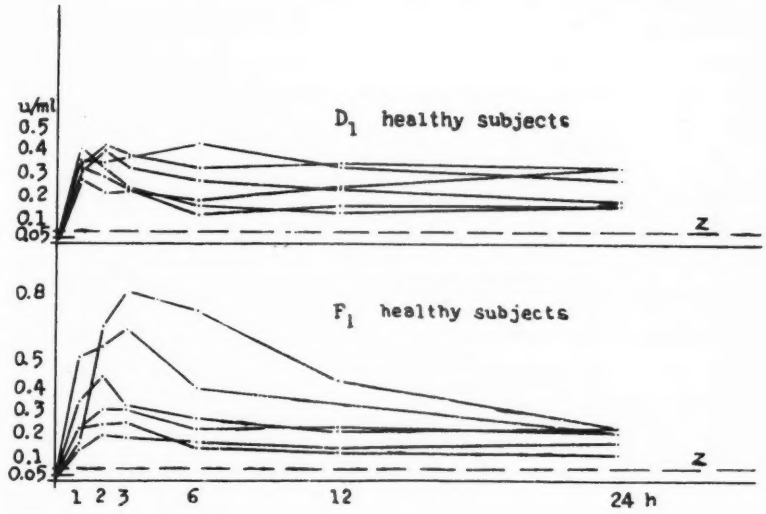

Fig. 6.

Figs. 5 and 6. — Individual penicillin concentrations during 24 hours in two series of five different adult hospital patients (fig. 5) and in two series of six healthy subjects (same persons) in light work (fig. 6) after a single intramuscular injection of 600,000 units of products F_1 and D_1 containing procaine penicillin G in aqueous suspension.

discarded subjects were essentially similar to those of their substitutes.

As shown in Fig. 4, products F^1 and D^1 containing procaine penicillin G in aqueous suspension gave mean concentration curves of a similar type for both the hospital patients and the test subjects in light work. The mean serum concentrations were approximately as high, and differences between products F^1 and D^1 were small. When also the standard deviations are taken into consideration, the two products must be regarded as similar when tested on each of the series of test subjects.

The mean maximum concentrations obtained for a hospital patient series with product F_1 was 0.40 and for another hospital patient series with product D_1 likewise 0.40 u./ml of serum. At 24 hours the mean values were 0.17 and 0.09 u./ml of serum, respectively. For persons occupied in light work the values for the two products (using the same series of persons for both products according to Boger) were 0.41 and 0.33 u./ml of serum at maximum and 0.19 and 0.24 u./ml of serum at 24 hours. Thus both preparations

maintained a higher serum penicillin concentration for 24 hours in working subjects than in hospital patients. This shows accordingly the increasing effect of movement on the serum penicillin level, which was observed by us already earlier (13).

When working subjects are used in tests of this kind, it is to be held in mind that the conditions must be similar not only on the test days but also in, at least, the preceding evening, for, as was earlier observed by us, physical exercise in the evening may have a marked effect on the serum penicillin values determined on the following day (13).

SUMMARY

Using the *S. lutea* method, the serum penicillin levels were determined with a view to:

1. effect of the penicillin dosage on the serum penicillin level,
2. duration of a serum penicillin level during work, and
3. serum penicillin levels obtained with two products using two different series of hospital patients and one series of working test subjects, the latter according to Boger's exchange system,

1. Using single intramuscular injections of 300,000, 600,000 and 1,200,000 units of a procaine penicillin G product in aqueous suspension, mean concentrations for hospital patients were 0.23, 0.40 and 0.88 u./ml of serum at maximum and 0.08, 0.17 and 0.25 u./ml of serum at 24 hours. With increasing penicillin doses, the serum penicillin level increased nearly proportionately to the size of the dose.

2. The following values were obtained for out-patients doing manual labour:

a) Using a single intramuscular injection of 600,000 units of a procaine penicillin G preparation in oily suspension, the mean maximum concentration was 0.98 u./ml of serum and the mean concentrations at 24, 48 and 72 hours were 0.44, 0.19 and 0.05 u./ml of serum. For a procaine penicillin G preparation in aqueous suspension the corresponding values were 1.32, 0.11, 0.08 and 0.04 u./ml of serum;

b) Using a single intramuscular dose of 2.4 million units of dibenzylethylenediamine dipenicillin G in aqueous suspension, a serum penicillin concentration exceeding 0.12 u./ml of serum was obtained for 21 days.

3. Tests were made with two procaine penicillin G products in aqueous suspension, administered in single intramuscular injections of 600,000 units into the gluteal region of hospital patients and according to Boger of test subjects occupied in light work under similar conditions, each hospital patient receiving an injection of only one product and the test subjects in light work an injection of each of the two products at separate times. Determinations of the mean serum penicillin concentrations for each product during 24 hours with series of five hospital patients and of six persons in light work gave similar values for both products in each series of test persons, with the exception that the working subjects had definitely higher mean concentrations at 24 hours than the hospital patients.

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FROM THE DEPARTMENT OF SEROLOGY AND BACTERIOLOGY, UNIVERSITY OF HELSINKI, AND FROM THE SURGICAL DEPARTMENT OF THE MARIA HOSPITAL, HELSINKI

ANTICOLIHEMOLYSIN (ACL), ANTISTAPHYLOLYSIN (ASTA) AND ANTISTREPTOLYSIN (AST) REACTIONS IN UROLOGIC INFECTIONS

by

OLOF WIDHOLM and R. POHJOLA

(Received for publication March 1, 1955)

During recent years, when studying the role of streptococci, staphylococci, pneumococci and E. coli in various diseases, the corresponding antihemolysin reactions have often been used as means of study. It is known that E. coli and Staphylococcus aureus are most common among the causative organisms in urinary infections. According to Campbell-Colston (1), Carrol stated the frequency of the former to be 26 and that of the latter 9 per cent. The present study was undertaken to elucidate the suitability and use of antihemolysin reactions (ACL and Asta) in urologic infections.

METHOD OF STUDY

The anticolihemolysin (ACL) determinations were performed according to the technique described by Widholm (2) and the antistaphylolysin (Asta) determinations according to the technique modified by him (3). The Kalbak (5) technique described by Oker-Blom (4) was used in antistreptolysin (AST) determinations. These tests were carried out in the Department of Serology and Bacteriology, University of Helsinki.

The bacteriologic urine analyses were performed in the State Serum Institute and partly in the Bacteriologic Laboratory of the City of Helsinki.

SERIES OF CASES

The series consisted of 135 hospitalized patients with unselected urinary infections. The main diagnoses were as follows:

	No. of Cases	Average Age in Years
Hypertrophia prostatae	46	69
Cystitis et pyelocystitis	18	53
Strictura urethrae	13	57
Tumor urogenitalis	22	64
Lithiasis	13	48
Tub. urogenitalis	8	36
Epididymitis non spesifica	3	55
Incontinentia urinae	2	33

The average age of the entire series was 59 years. Altogether 819 antihemolysin determinations were performed: 256 ACL, 284 Asta and 279 AST. An attempt was made to take the samples on an average at intervals of 10 days.

The bacteriologic analysis from sterile urine performed on 114 patients on the admission to the hospital showed sterile results in 39 cases (34 per cent). Seventy-five patients (66 per cent) had bacterial urinary infections caused by one organism in 57 cases (76 per cent), by two in 17 cases (23 per cent) and by three different organisms in only one case.

The bacterial findings varied greatly and corresponded to those mentioned previously in the literature. E. coli occurred in 39 (34 per cent) and Staphylococcus aureus in 24 (21 per cent) cases. Haemolytic streptococci were not found.

RESULTS

TABLE 1

ACL	0—199	200—399	≥400	%≥400	Total
Hypertrophia prostatae	60	20	16	17	96
Cystitis et pyelocystitis	10	11	16	43	37
Strictura urethrae	21	5	6	19	32
Tumor urogenitalis	18	15	18	35	51
Lithiasis	10	7	3	15	20
Tbc. urogenitalis	6	2	2	20	10
Epididymitis	4	1	3	38	8
Incontinentia urinae	1	1	—	—	2
Total	130	62	64	25%	256

TABLE 2

ASTA	0—0.99	1.0—1.99	2.0—	%=2.0	Total
Hypertrophia prostatae ..	59	20	28	26 %	107
Cystitis et pyelocystitis ..	16	17	3	8 %	36
Strictura urethrae	22	8	7	19 %	37
Tumor urogenit.	20	21	13	24 %	54
Lithiasis	13	10	2	8 %	25
Tub. urogenitalis	6	3	3	25 %	12
Epididymitis	5	—	4	44 %	9
Incontinentia urinae	1	—	—	—	2
Total	131 (51 %)	62 (25 %)	61 (24 %)	24 %	284

TABLE 3

AST	<200	≥200	Per Cent	Total
Hypertrophia prostatae	106	6	5 %	112
Cystitis et pyelocystitis	33	5	13 %	38
Strictura urethrae	19	3	14 %	22
Tumor urogenitalis	48	8	14 %	56
Lithiasis	19	8	19 %	27
Tub. urogenitalis	10	3	23 %	13
Epididymitis	8	1	11 %	9
Incontinentia urinae	1	1	50 %	2
Total	244	35	13 %	279

When comparing the relationships between the values for ACL, Asta and AST in the present study, it was found that ACL $\geq$ 400 occurred in 25 and Asta $\geq$ 2 in 24 per cent. As the groups formed on the basis of the main diagnoses were rather small, it did not seem necessary to discuss them separately. However, the high percentage for the increased ACL values was conspicuous in cystitis et pyelocystitis (43 per cent) and in tumore urogenitalis groups (35 per cent). High values for Asta occurred frequently in the following diseases: hypertrophia prostatae (26 per cent), tub. urogenitalis (25 per cent), tumor urogenitalis (24 per cent) and epididymitis (44 per cent). In the various disease groups, the percentage for the increased AST values was distinctly smaller than that of the two afore-mentioned titres. Lithiasis (29 per cent) and tub. urogenitalis (23 per cent) groups made, however, an exception.

To study the reliability of antihemolysin determinations we analyzed the cases with E. coli and Staphylococcus aureus cultivations. The corresponding values for ACL and Asta are to be seen in Table 4.

TABLE 4

ACL	E.coli in Urine 39 Cases		ASTA	Staphylococcus Aureus in Urine 24 Cases	
	No. of Cases	Per Cent		No. of Cases	Per Cent
0—199	4	10	0 —0.99	4	16
200—399	9	22	1.0—1.99	3	12
400—	26	68	2.0—	17	72

In these cases, the urinary E. coli infection was reflected by increased ACL values in 68 per cent. Correspondingly, the increased Asta values in 72 per cent reflected the staphylococcal infections.

It might be mentioned as a special observation that all three antihemolysin titres were never found to be increased simultaneously in parallel tests. The values for AST and Asta were increased simultaneously twice, for AST and ACL likewise twice, and for Asta and ACL 14 times.

RELATIONSHIPS BETWEEN ACL, ASTA AND AST VALUES BEFORE AND AFTER SURGICAL TREATMENTS

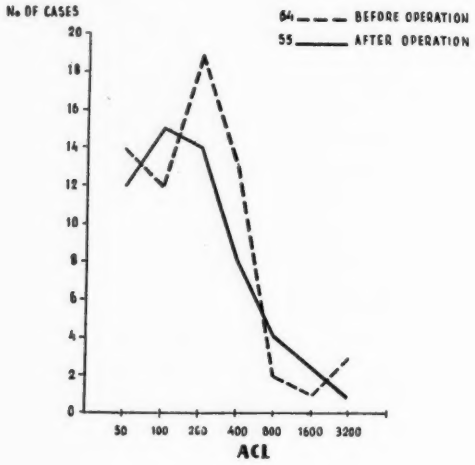

Fig. 1.

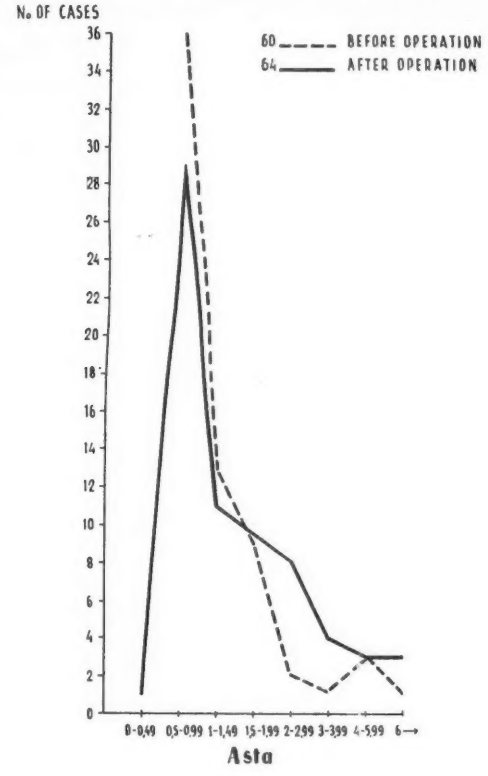
No OF CASES
60 BEFORE OPERATION
64 AFTER OPERATION
0-0,49 0,5-0,99 1-1,49 1,5-1,99 2-2,99 3-3,99 4-5,99 6→
Asta

Fig. 2.

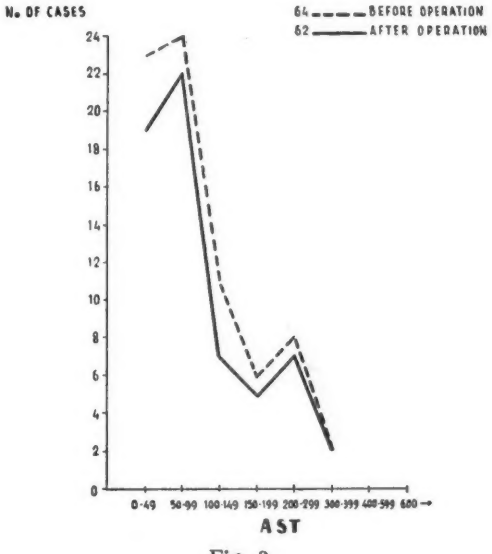
No OF CASES
64 BEFORE OPERATION
62 AFTER OPERATION
0-49 50-99 100-149 150-199 200-299 300-399 400-599 600→
AST

Fig. 3.

It can be seen in Fig. 1 that the post-operative values for ACL remained distinctly lower than those prior to the operation. This may be explained in view of the removal of infectious foci or through the improved drainage.

Fig. 2. shows that the Asta values were, on the contrary,

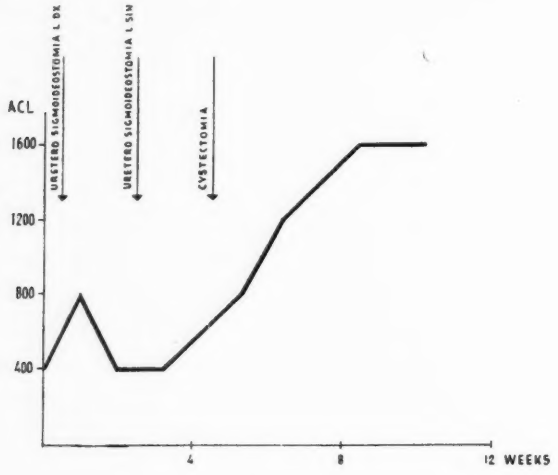

Fig. 4.

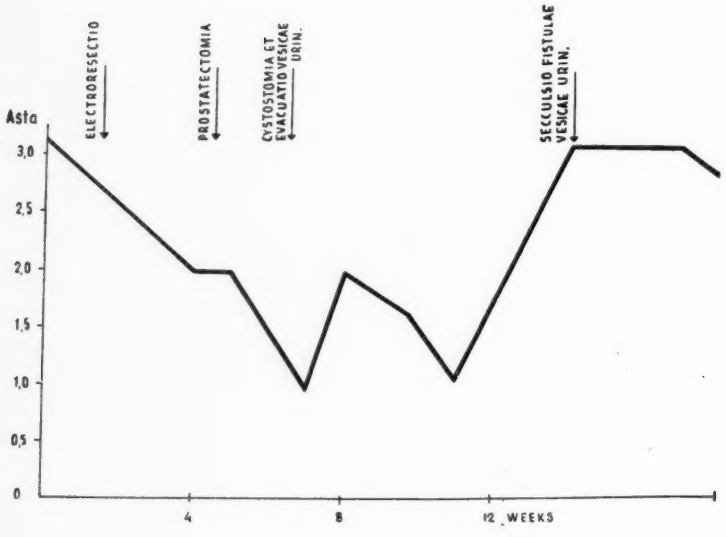

Fig. 5.

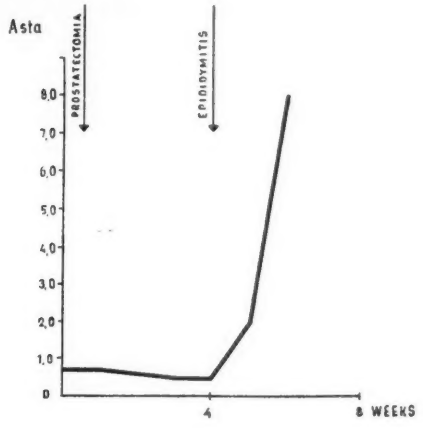

Fig. 6.

higher after the operation than before it. This became most evident in hypertrophia prostatae group in which 6 cases had increased values for Asta before the operation and 17 cases after it. On the other hand, surgical treatments caused no essential changes in the AST values (see Fig. 3).

The graph in Fig. 4 demonstrates the course of the ACL reaction in a group of patients. The course of the Asta reaction is represented graphically in Fig. 5 and 6.

URINARY BACTERIAL FLORA

The primary findings and the results after the treatment are shown in Table 5.

Table 5 shows that urinary infections still existed in 53 per cent of the cases in spite of therapy with sulpha drugs and antibiotics

TABLE 5

	Primary Findings		Findings after Treatment		
	No. of Cases	Per Cent	No. of Cases	Per Cent	
Sterile	19	34	41	47	
1 bact. species ..	57	76	34	66	
2 » » ..	17	24	10	20	53 %
3 » » ..	1	1	7	14	

directed on the basis of resistance determinations and in spite of an eventual surgical treatment. As compared with the primary findings, the increased percentage for the mixed infections was conspicuous after surgical treatments. Eleven different mixed infections caused by two species of bacteria and six combinations caused by three different bacteria occurred. The latter were the following.

Proteus mirabilis + Alcalescens dispar + Streptococcus faecalis
Proteus mirabilis + E. coli + Streptococcus faecalis
Proteus mirabilis + Staphylococcus aureus + Streptococcus faecalis
Staphylococcus aureus + Klebsiella + Streptococcus faecalis
Pseudomonas aeruginosa + Ballerup-Bethesda + Streptococcus faecalis
E. coli atypica + Staphylococcus aureus + Streptococcus faecalis

DISCUSSION

The patients examined were rather old, the average age being 59 years, and no striking antibody production was thus to be expected. Pure specific infections were relatively rare mixed infections forming the majority, which might also decrease the antibody production.

An evidence of the mutual independence of the different reactions is shown by the fact that two reactions were found to be increased simultaneously in only 18 cases of the 250 parallel determinations performed on all three reactions.

In the entire series, increased values for ACL were found in 25 and for Asta in 24 per cent. In specific urologic infections (Table 4), the corresponding figures for ACL and Asta were 68 and 72 per cent, respectively, which clearly reflected the specific sensitivity of the reactions. The parallel AST determinations, used in the control study, showed increased values in only 13 per cent in the entire series, which can be considered normal.

The detailed study of the present series (Tables 1, 2 and 3) revealed increased values for ACL ($\geq$400) in cystitis et pyelocystitis (43 per cent) and in tumor urogenitalis (35 per cent) groups. The former result is in correlation with the values reported by Widholm (2), viz. 64 per cent. This may be due to the fact that in these diseases infections have been chronic and occurring deep in the tissues.

Increased values for Asta (≥ 2) were found in the following groups: hypertrophia prostatae (26 per cent), tumor urogenitalis (24 per cent), tub. urogenitalis (25 per cent) and epididymitis (44 per cent). In general, the increased values occurred postoperatively as, *e.g.*, in hypertrophia prostatae group in which they were found in six cases prior to the operation and in 17 patients after it. Increased values for ACL occurred in 18 and 13 cases, respectively. A great danger of secondary staphylococcal infections is thus obvious. It also becomes clear on the basis of the results of urine cultures in which the frequency of infections caused by two or three different bacteria was distinctly higher in post-operative periods than prior to the operation.

SUMMARY

In 135 clinical cases with unselected urologic diagnoses, 256 ACL, 284 Asta and 279 AST determinations were performed. AST determinations were used as controls for the ACL and Asta reactions.

In 39 cases with E. coli infections ACL ≥ 400 in 68 per cent and in 24 infections due to Staphylococcus aureus Asta ≥ 2 in 72 per cent. The majority of the increased Asta values occurred post-operatively.

The frequency of mixed infections caused by two or three different bacterial species increased remarkably after surgical treatments.

In chronic urinary infections due to E. coli or Staphylococcus aureus, it is advisable to perform ACL and Asta determinations both before and after the operation, as they can throw light upon the quality of infections.

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FROM THE OUT-PATIENTS' DEPARTMENT FOR VENEREAL DISEASES AND THE LABORATORY OF SEROLOGY AND BACTERIOLOGY, KUOPIO

DOES TREATMENT WITH VITAMIN B_1 INFLUENCE SERORESISTANT SYPHILIS?

by

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Some references in the recent literature imply that in some cases of syphilis vitamin B_1 has the effect of rendering the Wassermann reaction temporarily negative (1, 3). Ferreira-Marques (3) treated 15 patients with strongly positive, »resistant» Wassermann and Kahn reactions with massive doses of vitamin B_1 (500 mg of »Benerva» *per os* daily for 10 days). The reactions turned entirely negative in 10 and weakened in 3 cases. In 2 cases no changes were noticed. Quantitative Wassermann and Kahn tests were not performed and the observation time was rather short.

The observations of the effect of Vitamin B_1 on the seroreactions of syphilis are of considerable interest. Because they are based on rather rough estimations we thought it justified to report our own results.

SERIES OF CASES AND METHODS

The patients were administered antisyphilitic treatment (principally, »adequate» arsenoxide and bismuth) for their seroresistance for years. Only 3 patients showed clinical symptoms of syphilis. The last antisyphilitic treatment (penicillin) was given on an average 9 months (4—18 months) prior to the beginning of vitamin B_1 therapy (Table 1).

TABLE 1

AGE, SEX AND DIAGNOSIS OF THE PATIENTS, QUALITY OF VITAMIN THERAPY AND ELAPSE OF TIME SINCE THE LAST ANTISYPHILITIC TREATMENT

No. of Case	Age	M	F	Diagnosis	Syphilis Diagnosed before Vitamin Therapy (months)	Last Antisyphilitic Treatment before Vitamin Therapy (months)	Vitamin Therapy (500 mg/day Perorally ×10)	Notes
1	47		1	Late latent syphilis	78	4	B_1 vit. »Star»	liquor WR —
2	45		1	Cardio-vascular syphilis	6	6	»	liquor WR —
3	43	1		»	9	7	»	liquor WR—§
4	45	1		Late latent syphilis	72	6	»	
5	55		1	»	48	9	»	liquor WR—§
6	52	1		»	84	18	»	liquor WR —
7	54	1		Cerebral syphilis	132	11	» §	liquor WR +
8	53		1	Late latent syphilis	60	12	»	
9	47	1		»	30	8	Benerva »Roche»	§
10	66	1		»	24	6	»	
11	71		1	»	66	6	»	liquor WR —
12	43	1		»	72	10	»	
13	41	1		»	54	13	»	
14	51		1	»	12	9	»	
15	39	1		»	66	10	»	
16	63	1		»	60	8	»	liquor WR —
17	59	1		»	30	9	»	
18	59	1		»	48	8	»	liquor WR —
19	55	1		»	36	8	»	§
20	63		1	»	120	12	»	§
21	63	1		»	36	9	»	§
22	54	1		»	12	10	»	liquor WR —
23	36	1		»	18	7	»	

§ = The patient was administered later Benerva »Roche» 500 mg/day perorally for ten days.
M = male
F = female

TABLE 2

THE FLUCTUATING TENDENCY OF SEROLOGIC TESTS DURING THE OBSERVATION TIME

No. of Case	Tested 179—120 Days before Vitamin Therapy			Tested 119—60 Days before Vitamin Therapy			Tested 59—30 Days before Vitamin Therapy			Tested 29—1 Days before Vitamin Therapy			Tested at the Beginning of Vitamin Therapy		
	WR	chWR	Kahn	WR	chWR	Kahn	WR	chWR	Kahn	WR	chWR	Kahn	WR	chWR	Kahn
2	5	4	4				5	5	5	5	5	5	5	5	5
3	5	5	5	4	5	5	4	5	5				5	5	5
4				2	3	3							4	4	4
5	3	3		2	3	3	2	2		2	2	4	3	3	4
7							0	2	3				0	1	3
9							3	3	5				3	3	5
10				2	3		2	3		2	3		2	3	4
11	4	5	5				4	5					4	5	5
12	2	2	3				0	1	2	0	2	3	0	1	2
13	0	1	2										0	2	3
14			4										3	3	3
15							0	0	2				0	1	3
16				1	1	2							0	0	2
17	5	5	5				5	5	5				4	5	5
18							0	0	3				0	0	2
19				4	4	5	4	4		3	4	5	4	4	5
20	2	2	4										2	2	4
21							3	3					2	3	4
23				0	1	2							0	1	2

[1] Cases No. 1, 6, 8 and 22 are not included in Table 2 as the serologic tests were performed before the observation time. In these cases, the tests performed at the beginning of the vitamin therapy can be seen in Table 3.

The quantitative Wassermann, cholesterol-Wassermann and Kahn tests were performed by diluting the serum in powers of 2. The fluctuating tendency of the tests before the vitamin therapy can be seen in Table 2. In Table 2, 0 indicates a negative result and the titers reflect the strength of a positive reaction. Figures 1—5 indicate the serum dilution in powers of 2, where the test still was positive.

Table 2 shows that the syphilis reactions of 5 patients (Case Nos. 9, 10, 11, 20 and 23) remained unchanged during the observation time before the therapy. In 7 patients (Case Nos. 2, 3, 4, 5, 13, 15, 19), the reactions showed a somewhat strengthening tendency

TABLE 3

SEROLOGIC RESPONSE OF CASES

No. of Case	Tested at the Beginning of Therapy			Tested 1—7 Days after Therapy			Tested 8—29 Days after Therapy			Tested 30—59 Days after Therapy			Tested 60-119 Days after Therapy			Tested 120—179 Days after Therapy		
	WR	chWR	Kahn	WR	chWR	Kahn	WR	chWR	Kahn	WR	chWR	Kahn	WR	chWR	Kahn	WR	chWR	Kahn
1	3	4	4	3	3											1	3	
2	5	5	5	4	4	5							5	5	5			
3	5	5	5	3	4	5				5	5		4	4	5			
4	4	4	4				3	3	4									
5	3	3	4	2	3	4							3	4	4			
6	3	3	5				1	3	4							1	2	4
7	0	1	3				1	2	2				0	1	1	0	0	3
8	0	3	4							0	2	4				1	1	5
9	3	3	5				2	3	4				1	4	4			
10	2	3	4				2	3	5	2	3	5	3	4	5			
11	4	5	5				4	5	5									
12	0	1	2	0	1	2				0	0	2				0	0	3
13	0	2	3										0	2	2			
14	3	3	3										2	2	3			
15	0	1	3				0	2	2				0	2	3	0	2	
16	0	0	2				0	1	2				0	2	2			
17	4	5	5				4	5	5	5	5					4	4	5
18	0	0	2				0	1	2				1	2	2			
19	4	4	5	3	4	5				4	4	5				3	4	5
20	2	2	4				2	2	4				1	2	3			
21	2	3	4				3	3	4	3	4							
22	5	4	4				5	4	4									
23	0	1	2				0	1	1				0	0	2			

and in 7 (Case Nos. 7, 12, 14, 16, 17, 18, 21) a tendency to weaken. The alterations in the reaction strength were, however, generally rather small. The strengthening was remarkable only in cases No. 4, 13 and 15, while cases 12 and 16 showed clearly weakening reactions. In our series, the earlier penicillin treatment did not thus seem to have notable effect on the reactions obtained during the observation time. This was in agreement with our knowledge on the effect of penicillin on seroresistant syphilis in general (2).

The serologic response of the patients after the vitamin therapy can be seen in Table 3.

DISCUSSION

The results listed in Table 3 are in disagreement with Ferreira-Marques's observations. We did not notice that the strongly positive Wassermann and Kahn reactions turned completely negative after the vitamin B_1 therapy. Even the slightly positive Wassermann or Kahn tests did not turn entirely negative during our study, while the slightly positive cholesterol-Wassermann turned negative in 3 cases (Nos. 7, 12 and 23). However, 2 of these patients (cases No. 7 and 12) showed a weakening tendency of the reactions even before the vitamin therapy. Prior to the vitamin therapy, the reactions remained unchanged in case No. 23 only.

Concerning the strength of the reactions, 15 cases (Nos. 1, 2, 3, 4, 5, 6, 7, 8, 9, 12, 13, 14, 19, 20 and 23) showed lower values in some respects. Three of them (cases Nos. 7, 12 and 14) had been weakening already before the vitamin therapy, while we had no data on the earlier tendencies in cases No. 1, 6 and 8. The reactions remained unchanged before the therapy in 3 cases (Nos. 9, 20 and 23) and tended to be strengthening in 6 cases (Nos. 2, 3, 4, 5, 13 and 19). Out of the last-mentioned 9 patients, only 4 (Nos. 3, 4, 5 and 9) showed remarkable decrease in the reactions, which was temporary in case No. 5.

After the vitamin therapy, the reactions became somewhat stronger in 6 cases (Nos. 10, 15, 16, 17, 18 and 21). Case No. 15 showed strengthening reactions even before the vitamin therapy and the reactions remained unchanged before the therapy in case No. 10. After showing a weakening tendency before the therapy, the reactions became stronger in 4 cases (Nos. 16, 17, 18, 21) after it.

In cases 10, 16, 18 and 21 remarkable strengthening was found in the reactions.

The reactions remained unchanged in case No. 11 in which no changes were noticed before the vitamin therapy either and in case No. 22 in which no data on the earlier reactions were available.

Accordingly, we cannot agree with the opinion that the vitamin B_1 therapy has a weakening effect on the positive Wassermann and Kahn reactions. The 4 cases in our series, in which the vitamin therapy clearly seemed to weaken the reactions, corresponded to the other 4 in which became evident that the vitamin therapy had a distinctly strengthening effect on the reactions. The slight alterations in the reactions during the observation time may be due to other reasons, such as technical errors, *etc.*

SUMMARY

Twenty-three patients with seroresistant syphilis were administered vitamin B_1 perorally. There was no evidence that the vitamin B_1 therapy would influence the serologic tests for syphilis.

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AUS DER KINDERKLINIK DER UNIVERSITÄT HELSINKI, UND AUS DEM STAATLICHEN SERUMINSTITUT, HELSINKI

ÜBER DIE PATHOGENITÄT DER ESCHERICHIA COLI 0—26: B: 6

von

ILARI RANTASALO, SEPPO TALANTI and NIILO HALLMAN

(Am 14. 4. 55 bei der Schriftleitung eingegangen)

In zahlreichen Untersuchungen hat man festgestellt, dass Esch. coli 0—26 dieselbe pathogenetische Bedeutung für die Gastroenteritis des Kleinstkindes hat, wie die Typen 0—111, 0—55, und 0—86. In den neuesten Untersuchungen hat man festgestellt, dass nach Abklingen der durch alle diese Typen verursachten Durchfälle sich spezifische Agglutininen gebildet hatten. Bei der Hälfte der durch Esch. coli 0—26: B: 6 erkrankten Kinder, bei denen dieser Stamm auch nachgewiesen wurde, konnte man diese Agglutininen feststellen (5).

In Fütterungsversuchen mit den Typen 0—111 (14, 11, 10, 3) 0—55 (2) und 0—86 (3) wurden bei Erwachsenen typische Gastroenteritiden hervorgerufen, während die gleichen Proben mit den Stämmen vom Typ 0—26 negativ blieben (3).

Für die Tierversuche hat man Mäuse, Mäusejungen, Kücken, Kaninchen, Meerschweinchen, Affen, Katzenjungen und Kälber (Näheres darüber bei 4) verwendet. Die Resultate sind überhaupt negativ gewesen. Bei Kälbern hat man jedoch milde Diarrhoe mit dem Typ 0—111 (1) observiert, die Versuche konnte man jedoch nicht bestätigen (4). In diesem Zusammenhange kann gesagt werden, dass alle die angeführten Bakterientypen in den Kühen gefunden wurden und weiter die Typen 0—26 (16) und 0—55 (19) in den an Durchfällen erkrankten Kälbern.

In den Fällen, wo die Typen 0—26 und 0—55 den Meerschweinchen in die Harnblase injiziert wurden, fand man an der Schleimhaut der Harnblase deutliche Entzündungsherde. Die Entzündungsherde waren in den Fällen, wo den Versuchstiere die Typen 0—111 und 0—26 injiziert worden waren, merklich schwächer.

Von den positiv ausgefallenen Tierversuchen (15) mögen noch jene mit Mäusen erwähnt werden, die zeigten, dass Dyspepsie-coli erheblich mehr Toxine bilden, die bei den Mäusen unter Manifestation von cerebralen und enteralen Symptomen zum Tode führten.

Da Esch. coli 0—26: B: 6 nach den bisherigen Untersuchungen (17, 8, 7) hier in Finnland das am meisten verbreitete Dyspepsie-coli darstellt, haben wir uns besonders für diesen Typ interessiert, mit dem man bisher anderswo erst wenige Tierversuche angestellt hat. Um die Pathogenität dieser Typen aufklären zu können, haben wir Tierversuche mit Jungschweinen, Katzenjungen und Hühnerembryonen vorgenommen. Ausser diesen Tierversuchen probierten wir die Infektiosität von Esch. coli 0—26 an einigen schwer oligophrenen Säuglingen und Kleinstkindern.

VERSUCHE MIT JUNGSCHWEINEN

Für diese Versuche verwendeten wir Tiere von 3 verschiedenen Würfen. Nach der Entwöhnung vom Mutterschwein wurden die Tiere gesondert untergebracht und mit Kuhmilch, Zwiebackbrei, Hafermehl und mineralischer Kost gefüttert. Sämtliche Versuchtiere waren gesund und in gutem Allgemeinzustand. Vor Beginn der Versuche wurden die Stühle der Tiere auf Dyspepsie-coli untersucht und die Körpertemperatur regelmässig geprüft. Zur Verabreichung der Bakteriensuspension verwendeten wir gewöhnlich 24 Stunden alte Bouillonkulturen, für die man 0,5—1,0 Milliarden Bakterien pro ml berechnet.

Serie 1. — 3 Stück 6 Wochen alte Jungschweine.

Eines der Tiere diente als Kontrolltier und erhielt reine Bouillon per os. Das zweite Jungschwein wurde mit einer Aufschwemmung von Esch. coli gefüttert, dessen Stamm wir aus Durchfallstühlen von kranken Jungschweinen isoliert hatten. Das dritte Jungschwein erhielt Esch. coli 0—26. Pro Tag erhielt somit das erste Tier fortlaufend reine Bouillon und die anderen Tiere Kulturen in der Menge von 100 ml (=50—100 Milliarden Bakterien). Während der ersten Tage des Versuches war das Allgemeinbefinden aller Versuchstiere gut. 7 Tage nach Versuchsbeginn erhielt

auch das Kontrolltier Kulturen von Esch. coli 0—26. Das Befinden des Tieres blieb trotz dieser Massnahme gut. Nach 8 Tagen konnte man aus den Stühlen des letzten Jungschweines Esch. coli 0—26 absondern, die sich auch noch in den 2 folgenden Wochen bei den Kontrollen feststellen liessen.

Das Befinden sämtlicher Versuchstiere blieb während der Behandlungsdauer gut. Die Fresslust hielt sich normal, die Stühle waren geformt und die Gewichtszunahme zufriedenstellend.

Serie 2. — 3 Stück 3 Wochen alte Jungschweine.

Allgemeinzustand der Tiere merklich schwächer als bei den Tieren der ersten Versuchsreihe.

Die per os gegebene Bouillon betrug 50 ml pro Tier und Tag. Wie bei der ersten Versuchsreihe erhielt eines der Tiere zur Kontrolle reine Bouillon. Das zweite Tier erhielt eine Aufschwemmung von Esch. coli 0—26 und das dritte Tier eine Kulturaufschwemmung eines Coli-Stammes, den wir aus den Durchfallstühlen eines an typischer Ferkeldiarrhoe erkrankten Tieres gewonnen hatten. Das mit Esch. coli 0—26 gefütterte Tier nahm am 3. Tag keinerlei Nahrung mehr zu sich und ging am folgenden 4. Tag zugrunde. Das Kontrolltier erkrankte ebenfalls an heftigen Durchfällen, die Körpertemperatur sank unter die Norm und das Tier zeigte starke Cyanose. Am 5. Tag ging auch dieses Tier zugrunde. Bei der Sektion fanden wir in beiden Fällen typische Zeichen einer Ferkeldiarrhoe und in den Bakterien der Organe fanden wir Esch. coli, die aber nicht zum Typ 0—26 gehörten. Das Tier, welches mit dem Stamm gefüttert wurde, den wir aus dem Stuhl einer Ferkeldiarrhoe gewonnen hatten, lebte am längsten. Erst am 6. Tag nach Versuchsbeginn nahm es keinerlei Futter mehr an, wurde cyanotisch und ging am 9. Tag nach dem Versuchsbeginn ein. In diesem Falle ergab die Sektion eine Pneumonie. In den Lungen fanden wir Streptococcen, jedoch keine Esch. coli.

Serie 3. — 4 Stück 4 Wochen alte Jungschweine.

Um die Wirkung der Toxine darzustellen, wurde den Versuchstieren 10 ml 5 Tage alte Bouillonkultur, von der die Bakterien abfiltriert worden waren, intraperitoneal injiziert. Eines der Versuchstiere erhielt ein Filtrat von »Normal»-Coli, das zweite erhielt ein Filtrat von Esch. coli 0—26 und das dritte Versuchstier erhielt ebenfalls ein Filtrat von Esch. coli 0—26, doch war die Kultur vor der Filtrerung mit Aqua dest. behandelt worden. Das vierte Versuchstier erhielt den mit Aqua dest. behandelten Filtratrückstand der Kultur, mit der das zweite Versuchstier behandelt wurde. Die Beobachtungszeit dieser Tiere betrug 10 Tage. Innerhalb dieser Zeitspanne blieben sämtliche Versuchstiere ohne Anzeichen einer Erkrankung.

Zusammenfassend kann von den Versuchen mit den Jungschweinen gesagt werden, dass Esch. coli 0—26: B: 6 per os verabreicht oder dessen Toxine intraperitoneal injiziert keine Störungen verursachten. Ausserdem beweist Versuchsreihe 2, aus der sämtliche Versuchstiere zugrunde gingen, dass sowohl die von

anderen Ursachen herrührende schlechte Kondition der Tiere als auch die zum Tode führenden Krankheiten trotz der verminderten Widerstandskraft der Tiere zu keiner Überschwemmung der gleichzeitig per os gegebenen Bakterien in den Organen geführt hat.

VERSUCHE MIT JUNGEN KATZEN

Unsere Versuchsreihe umfasste 2 Würfe von je 3 Tieren, von denen der erste Wurf drei Wochen und der zweite Wurf sechs Wochen alt war. Die Tiere erhielten mit ihrem Futter eine Menge von 1—10 ml einer 1 Tag alten gewöhnlichen Kultur Esch. coli 0—26: B: 6. Zeichen einer Erkrankung konnten während der ganzen Versuchszeit nicht festgestellt werden.

VERSUCHE MIT HÜHNEREMBRYONEN

Um die Toxine der Bouillonkulturen und ihre Wirkung aufzuklären, injizierten wir das Filtrat einer Kultur Esch. coli 0—26: B: 6 in den Dottersack, haben jedoch keinerlei Entwicklungsstörungen an den Hühnerembryonen feststellen können.

NÄHRVERSUCHE AN KLEINKINDERN

Versuch 1. — 16 Monate altes Mädchen.

Klinische Diagnose: Microcephalus, Eclampsia.

Das Kind erhielt mit der Milch 1 ml einer Kultur von Esch. coli 0—26 (= etwa 0,5 Milliarden Bakterien).

Für die Kultur verwendeten wir einen alten Laboratoriumsstamm, den wir vor 3 Jahren aus Dänemark erhalten haben. Versuchsdauer war 3 Wochen.

Während dieser Zeit keine Durchfälle. Die Gewichtszunahme war normal. Esch. coli 0—26 konnten aus dem Darm nicht gewonnen werden.

Versuch 2. — 10 Monate alter Knabe.

Klinische Diagnose: Laesio cerebri, Furunculosis, Status post gastroenteritidem.

Während der Gastroenteritis wurden Dyspepsie-coli nicht gefunden.

Das Kind erhielt 1 ml einer aus dem Stuhl eines durchfallkranken Kindes gewonnen Kultur Esch. coli 0—26. Nach 2 Tagen war die Stuhlkultur des Kindes auf Esch. coli 0—26 positiv. Versuchsdauer 2 Wochen. In dieser Zeit waren die Stühle fest geformt, es zeigten sich keine auffallenden Störungen in der Verdauung und die Gewichtszunahme war zufriedenstellend.

Versuch 3. — 1½ Monate alter männlicher Säugling.

Klinische Diagnose: Meningocele, Hydrocephalus.

Das Kind erhielt 1 ml Kultur von einem frisch gewonnenen Stamm Esch. coli 0—26.

Allgemeinzustand blieb unverändert, es entstanden keine Durchfälle. Stuhl auf Esch. coli 0—26 negativ.

Versuch 4. — 14 Monate alter Knabe.

Klinische Diagnose: Laesio cerebri, Eclampsia.

Wie im Versuch 3 Verabreichung von 1 ml Kultur frisch gewonnener Esch. coli 0—26.

Stuhlkultur auf Esch. coli 0—26 nach 8 Tagen positiv.

Keine Durchfälle, gutes Allgemeinbefinden.

Über die Versuche mit Kindern kann zusammenfassend gesagt werden, dass in keinem der Fälle Esch. coli 0—26: B: 6 zu Durchfällen führte, obwohl in zwei Fällen die Stuhlkulturen positiv wurden.

SCHLUSSBETRACHTUNGEN

Auf Grund der Ergebnisse unserer Versuche könnte man denken, dass Esch. coli 0—26: B: 6 für Jungtiere und Kleinkinder ebenso apathogen sind wie auch die meisten anderen Esch. coli-Stämme. Freilich beweist das Fehlen der Pathogenität von Esch. coli 0—26: B: 6 für das Tier keineswegs die Apathogenität in Bezug auf den Menschen. Das übliche Auftreten von Esch. coli 0—26 in den Durchfällen von Kleinkindern (8, 17) wie auch die Feststellung von Ketteninfektionen in Anstaltabteilungen (13, 9) sowie der in der Einleitung erwähnte Hinweis auf die Feststellung spezifischen Agglutininen bei den von Durchfallerkrankungen genesenen Kindern sprechen deutlich für eine Kleinkinderpathogenität von Esch. coli 0—26: B: 6.

Dyspepsie-coli konnte man nach Angaben einiger anderer Referenten bei durchfallkranken Kindern nicht öfter feststellen als bei gesunden Kindern (12, 18). Somit scheint es lokal bedingte Variationen zu geben. Aus diesen Gründen und entsprechend den E gebnissen unserer eigenen Untersuchungen kann man annehmen, dass zu der Entstehung einer Infektion mit Esch. coli 0—26 ein Zusatzfaktor notwendig ist.

ZUSAMMENFASSUNG

Es wurden Versuche mit Esch. coli 0—26: B: 6 durchgeführt, indem man sie per os jungen Schweinen und Katzen verabreichte. Erkrankungen, die man als von Esch. coli 0—26: B: 6 verursacht erklären könnte, sind nicht aufgetreten. Man wollte die Wirkung der Toxine der Bakterienkulturfiltrate prüfen und injizierte es Jungschweinen intraperitoneal und Hühnerembryonen in den Dottersack. Toxische Symptome zeigten sich keine. Die Zuführung von Bakterienkulturen (etwa 0,5 Milliarden Bakterien) bei 4 Kleinkindern verursachte ebenfalls keine Erkrankung, obwohl in 2 Fällen in den Stühlen Bakterien nachgewiesen werden konnten.

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FROM THE DEPARTMENT OF SEROLOGY AND BACTERIOLOGY, UNIVERSITY OF HELSINKI, AND FROM THE WIHURI RESEARCH INSTITUTE, HELSINKI

STUDIES CONCERNING A FIBRINOLYTIC ENZYME PREPARATION

by

EERO ESTOLA, JAAKKO ELO, and V. J. KÄRKKÄINEN

(Received for publication April 16, 1955)

When studying the anticoagulating ability of saline solutions prepared from fungi (1) it was found that four species stood out as showing this power to a greater degree than the rest. Whole blood was used at this stage of the study. Further researches proved the comparatively frequent occurrence of such agents in the fungus flora even though, with the technique then used, they could be demonstrated in a few species only. In the common species *Tricholoma equestre* this property was constantly marked. On a closer examination of the extract from *T. equestre* its anticoagulative activity turned out to be due to its fibrinogenolytic power.

The aim of subsequent researches was to purify the extract and to study its properties as well as its effect on other proteins.

Preparation and Purification. — After collection the fungi were dried at room temperature. The ground fungi were suspended in saline in the proportion of 1 to 10. The suspension was incubated for 2 or 3 hours at 37° C and then overnight at room temperature. Filtration through surgical gauze removed the insoluble sediment and gave an opaque oily solution of a grey-brown colour. When clarified by means of Chamberland filter the extract became reddish-

brown while the activity remained unaffected. It was possible to attain a certain degree of lightening by adding 20% zinc acetate solution in the proportion of 1 to 10, by separating the precipitate in a centrifuge, and by dialysing the active supernatant against distilled water at +4° C for several days. After lyophilisation the activity remained unaffected. Below, the letters T.e. stand for this powder.

The Effect of the T.e. Preparate on Some Proteins. — The effect of the T.e. preparate on fibrinogen, fibrin and thrombin, which participate in the process of clotting, as well as on gelatin and casein was studied by comparing it with some commonly known proteolytic enzymes.

Reagents. — Trypsin (Difco, 1 : 250), Papain (Difco), Tyrosinase (Worthington, 3—4 Cresolase U/mg. and 4.5 Catecholase U/mg), Streptokinase (Varidase, kindly supplied by Lederle Lab.), Plasminogen (Hum. plasma Fr. III, kindly supplied by Miss Eeva Alameri, M. Sc. in Chemical Engineering, Finnish Red Cross), Fibrinogen (Armour bov. Fr. I), Thrombin (Hoffmann-La Roche).

Fibrinogen. — The method of Ungar & Mist, somewhat modified, was employed in the study of fibrinolysis (7). A 0.1 per cent solution from T.e., trypsin and tyrosinase each, and a 1 per cent suspension from papain, were all prepared in saline. Further dilutions in powers of 2 were made in saline. The test was carried out by incubating 0.5 ml. of the enzyme dilutions along with an equal quantity of fibrinogen solution (0.25 per cent in the veronal buffer of Michaelis at pH 7) in water bath at 37° C for 30 minutes.

From Varidase a solution was made containing 2000 U. of streptokinase per ml., and of this further dilutions in powers of 2 were made. Streptokinase activated plasminogen was prepared by mixing 0.1 ml. of each Varidase dilution with 0.5 ml. of plasminogen (0.25 per cent in saline). This series of dilutions was incubated with a 0.25 per cent fibrinogen solution like the other enzymes.

After the solutions were removed from the water bath, 0.02 ml. of thrombin solution (0.25 per cent in saline) was added into each tube. After 10 minutes those dilutions were checked in which the enzyme had inhibited the formation of fibrin.

TABLE 1

The Fibrinogenolytic Power of the Enzyme Preparates		1/1	1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256	1/512
T.e.	1 mg./ml.	+	+	+	+	+	+	+	+	±	—
Trypsin	» »	+	+	+	+	+	+	—	—	—	—
Tyrosinase	» »	+	+	+	+	—	—	—	—	—	—
Papain	10 mg./ml.	+	+	+	±	±	±	—	—	—	—
Streptokinase activ. plasminogen		+	+	+	+	+	—	—	—	—	—

Symbols: + = no coagulation; — = coagulation.

The fibrinogenolytic activity of T.e. is clearly stronger than that of the other enzymes.

Fibrin. — In test tubes, 0.5 ml. of fibrinogen solution (0.25 per cent in the veronal buffer of Michaelis at pH 7) was coagulated into fibrin by means of 0.02 ml. of thrombin. Into each tube was added 0.5 ml. of the enzyme dilutions. Digestion was allowed to take place in water bath at 37° C. The tubes were shaken every 30 minutes. After four hours' incubation they were removed from the water bath and the results were read.

TABLE 2

The Fibrinolytic Power of the Enzyme Preparates		1/1	1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256	1/512
T.e.	1 mg./ml.	+	+	+	+	+	±	—	—	—	—
Trypsin	» »	+	+	+	±	—	—	—	—	—	—
Tyrosinase	» »	+	±	—	—	—	—	—	—	—	—
Papain	10 mg./ml.	—	—	—	—	—	—	—	—	—	—
Streptokinase activ. plasminogen		+	+	+	+	+	±	—	—	—	—

Symbols: see Table 1.

In fibrinolysis the activity of T.e. is stronger than that of trypsin and tyrosinase, and about equal with the streptokinase activated plasminogen employed.

Thrombin. — Difficulties in the study of the activity on thrombin are presented by the fact, that the enzymes also break down fibrinogen, which is the indicator of thrombin. For this reason

the proportion of fibrinogen to the enzyme was greater than above; moreover, by means of controls it was made sure that the enzymes used did not break down fibrinogen during the examination. For the examination 0.02 ml. of the T.e. and trypsin dilutions together with an equal volume of 0.25 per cent thrombin solution was incubated at 20° C for 24 hours. Next, 1 ml. of fibrinogen solution (0.25 per cent) was added into each tube. An identical series, in which the enzyme was added into thrombin immediately before fibrinogen, served as a control. The result was read after 10 minutes at 20° C.

TABLE 3

Inactivation of Thrombin by T.e. and Trypsin						
Coag. power of thrombin after incub. of 24 h. with	1/1	1/2	1/4	1/8	1/16	1/32
T.e. ..... 1 mg./ml.	+	+	+	+	±	—
Trypsin ..... » »	+	+	+	—	—	—
Contr.						
T.e. ..... 1 mg./ml.	—	—	—	—	—	—
Trypsin ..... » »	—	—	—	—	—	—

Symbols: see Table 1.

The proteolytic effect of T.e. on thrombin seems to be somewhat stronger than that of trypsin.

Casein. — The effect on casein was studied according to Kunitz (2) by incubating the enzyme-substrate mixture in water

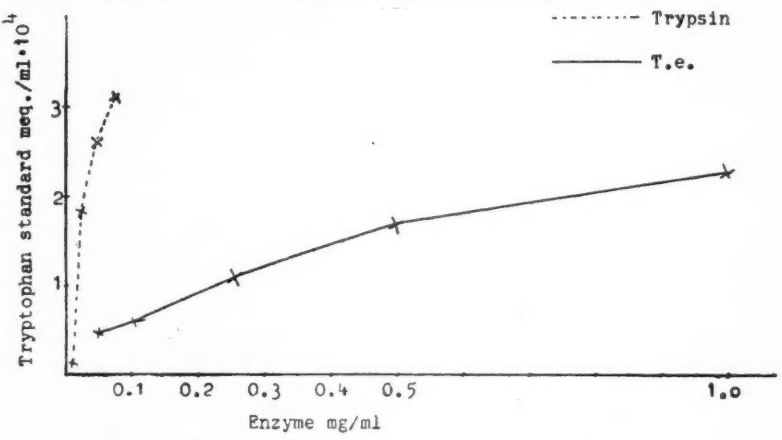

Fig. 1. — Digestion of Casein by T.e. and Trypsin.

bath at 37° C for 20 minutes. The non digested protein was precipitated by means of 5% thrichloracetic acid. The amount of split products in the supernatant was determined colorimetrically by means of the phenol reagent of Folin-Ciocalteau; the results were compared with tryptophan standard. (Fig. 1).

The proteolytic effect of T.e. on casein is definitely weaker than that of trypsin.

Gelatin. — The formol titration method modified by Kunitz (2) was used in the study of the effect on gelatin. The enzyme was allowed to act on the gelatin solution in water bath at 37° C. After 20 minutes, formaldehyde, indicator and sodium hydroxide were added to the reaction mixture. The surplus of NaOH was titrated with HCL.

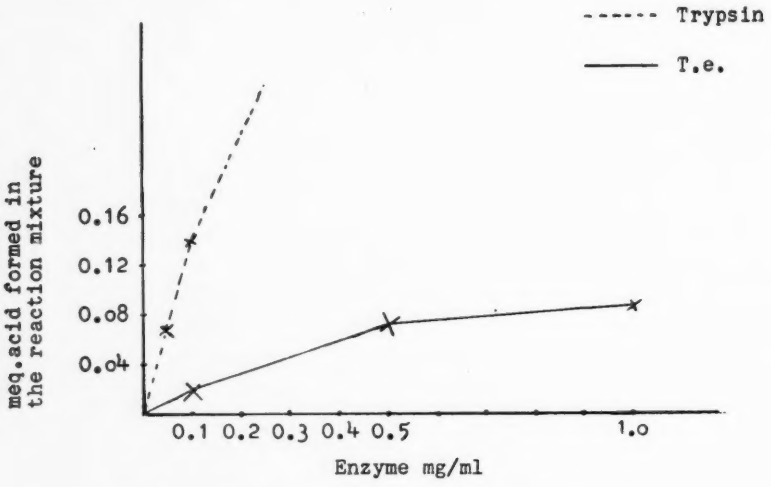

Fig. 2. — Digestion of Gelatin by T.e. and Trypsin.

The proteolytic effect of T.e. on gelatin is definitely weaker than that of trypsin.

Effect of Heating and pH on the T.e. Preparate. — In order to study the termolability of the T.e. preparate the preparate was incubated in 0.1 per cent solutions at different levels of temperature for half an hour. After that the fibrinogenolytic power of the solutions was determined according to Ungar & Mist as above.

TABLE 4

The Effect of ½ Hour's Heating on the T.e. Preparate (Ungar & Mist)		1/1	½	¼	1/8	1/16	1/32	1/64	1/128	1/256
Before heating		+	+	+	+	+	+	+	+	—
After heating for ½ hour at	56°C	+	+	+	+	+	+	+	—	—
	60°C	+	+	+	+	±	—	—	—	—
	65°C	—	—	—	—	—	—	—	—	—

Symbols: see Table 1.

It will be seen that the activity of the preparate begins to diminish at 56° C; total loss of activity occurs at 60—65° C.

For the purpose of studying the share of pH in fibrinolysis suitable dilutions of T.e., trypsin, tyrosinase, and papain were prepared in several buffers of varying pH. The dilutions were tested for fibrinolysis by means of the fibrin plate method of Permin (4). Optimum effect from all enzymes was obtained on the acid side. The fibrinolytic power of tyrosinase and papain diminished noticeably towards the alkaline direction. The effect of T.e. and trypsin also weakened even though much less.

Tyrosinase Content of the T.e. Preparate. — Since certain fungi are rich in tyrosinase, which is known to break down fibrin, fibrinogen and thrombin (5, 6), a study was made of how the T.e. preparate, in the concentration of 1 mg./ml., would cause oxidation of tyrosin. Tyrosinase solution (1 mg./ml.) was used as control. The test was carried out by adding 0.02 ml. of the enzyme solution to 1 ml of tyrosin solution (0.2 mg./ml.) in a test tube. After 24 hours at 37° C the tyrosin that had been treated with the T.e. solution was still colourless whereas the tyrosinase control had turned brown.

Electrophoretic Examination of the T.e. Preparate. — For this test, 30 mg. of the T.e. preparate was dissolved in 0.2 ml. of saline and the insoluble sediment was removed by centrifuging. In a semipreparative paper electrophoresis using 0.05-n medinal veronal buffer (pH 8.6), the dissolved part was found to split into two fractions. The position of these fractions can be seen in Fig. 3, which shows the grouping of the proteins of the sera in identical circumstances.

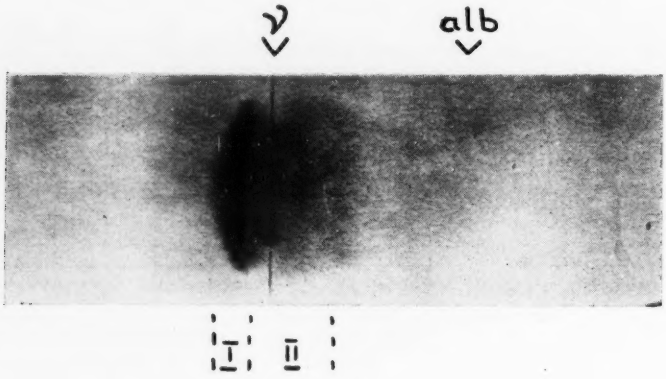

Fig. 3.

The electrophoresis strip was cut as shown in Fig. 3 and the fractions were washed in 1 ml. of saline. Fraction I showed the greater part of the fibrinolytic activity. Fraction II was also active, though to a smaller degree.

The Ribonuclease Activity of the T.e. Preparate. — Mäkitalo (3) has shown the remarkable ribonuclease content of *Tricholoma equestre*. He kindly determined the ribonuclease activity of the preparate. Considerable increase in the activity was recorded after purification as compared with the crude extract. The ribonuclease activity of fraction II seems to be somewhat greater than that of fraction I.

DISCUSSION

The enzymatic character of the T.e. preparate is shown by its high degree of activity and its response to heating. The fibrinolytic effect of the T.e. preparate is stronger than that of trypsin, which, on the other hand, clearly surpasses the proteolytic activity of T.e. on such proteins as casein and gelatin. This seems to imply that, in fibrinolysis, the effective agent in T.e. is not likely to be a proteolytic enzyme; instead, the effect of the T.e. preparate is possibly due to its capability of breaking up the bridges between long-chain molecules.

As has been mentioned above the ribonuclease content of *Tricholoma equestre* is high. The purification procedure used for the T.e. preparate also caused an enrichement of its ribonuclease activity.

Examination by electrophoresis resulted in the division of the T.e. preparate into two fractions. One of these possessed distinctly higher fibrinolytic power, while the other showed somewhat greater ribonuclease content. Sufficiently accurate fractioning for the separation of these enzymes was not as yet feasible owing to the small quantity of the preparate available.

It was possible to show with certainty that the effective agent in the preparate was not tyrosinase, of which comparatively large quantities are known to occur in fungi, and which is also known to have a proteolytic effect on fibrin, fibrinogen and thrombin. It should be noted that the T.e. preparate could not bring about any distinguishable oxidation of tyrosin though it caused a far stronger fibrinolysis than the tyrosinase preparate that was used as a control.

SUMMARY

A study was made of the purification and properties of an enzyme preparate obtained from the fungus *Tricholoma equestre.* Its anticoagulating activity was found to be due to its effect on fibrinogen and thrombin; moreover, it was found to digest fibrin and, though weakly, casein and gelatin. The preparate contains considerable quantities of an enzyme capable of splitting up ribonucleic acid. It has no monophenoloxydase effect.

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FROM THE DEPARTMENT OF SEROLOGY AND BACTERIOLOGY, UNIVERSITY OF HELSINKI

PHYTAGGLUTININS IN LICHENS

by

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Since Renkonen observed in 1948 (4) that the seeds of certain species of Leguminosae contain phytagglutinins selectively agglutinating different blood groups, interest in research in this branch has revived. Boyd and Reguera (1) made an extensive investigation into phytagglutinins contained in seeds and found some new species, mostly containing an anti-A property. Krüpe (3) was the first to show the agglutinins of the incomplete type present in plant extracts. Besides those found in extracts made from seeds, phytagglutinins have been shown in fungi also (2).

Lichens have not been studied in this sense, as far as we know. Since they are symbioses of fungus and alga they seemed likely to be interesting objects of investigation. This prompted us to carry out similar experiments with one hundred Finnish lichen species. The material was collected prior to 1950 and kept dry at room temperature. It was identified by Prof. E. Häyren, Dr. V. Räsänen and Mr. L. Fagerström, M.Sc. For practical reasons, small-sized lichen species were excluded from the study.

TECHNIQUE

The technique applied in the preparation of the extracts was approximately the same as in the study of seeds. The lichens were crushed by grinding in a mortar or, if this failed, by chopping them. The powder obtained was extracted with saline (1 : 10) and the

suspension was left in an incubator (+ 37° C) overnight. With a tight-fitting cellulose wadding piston the lichen mass was pressed to the bottom of the test tube, leaving a clear filtrate on the surface. This filtrate was used for the investigations.

One drop of the filtrate to be studied and an equal amount of 5 per cent red cell suspension (in saline and in compatible serum) were placed in 50 × 4 mm test tubes. The tubes were allowed to stand for 1—2 hours at room temperature, then read by microscope. The four test cells were employed, permitting selective testing with regard to blood groups ABO, MN, Pp and Le.

RESULTS

With 44 out of 100 species the filtrate acting on cells suspended in saline gave a more or less clear haemolysis, with an incubation period of 2—3 hours. With 1-hour incubation, only 4 species induced a complete and 8 species a partial haemolysis. With cells suspended in serum there was no haemolysis whatever, but agglutination too failed to appear with 3 saline-agglutinating species.

The 92 species (92 per cent) enumerated in table 1 proved negative as regards agglutination both in saline and in serum:

TABLE 1

NONAGGLUTINATING SPECIES OF LICHEN

Dermatocarpon miniatum	Gladonia deformis
Sphaerophorus fragilis	» digitata
Diploschistes scruposus	» fimbriata f. simplex
Leptogium saturninum	» Floerkeana
Lobaria pulmonaria	» furcata
Nephroma parilis	» » var.palamea
Peltigera rufescens	» gracilis
» scaprosa	» » var.chordalis
» variolosa	» » var.dilatata
Lecidea fuscoatra	» rangiferina h
Biatora granulosa	» silvatica (h)
Psora demissa	» squamosa
Cladonia alpestris (h)[1]	» turgida
» alpicola (h)	» uncialis
» amaurocrea	Stereocaulon denudatum
» cenotea (h)	» » var.digitata (h)
» coccifera	» paschale
» cornuta	» subcoralloides Nyl.

[1] h = hemolysis
(h) = partial hemolysis

Gyrophora deusta
» hirsuta
» polyphylla
» vellea
Umbilicaria pustulata
Pertusaria discoidea
Parmularia muralis (Squamaria saxicola)
Lecanora cenisea
Ochrolechia androgyna
» tartarea
Haemmatoma ventosum
Parmeliopsis ambigua
Parmelia centrifuga
» conspersa
» fuliginosa
» incurva
» olivacea
» omphalodes f. grisea
» panniformis
» physodes
» saxatilis
» stenophylla
» stygia
» sulcata
» tubulosa
Cetraria clorophylla
» glauca
» islandica
Cetraria tenuifolia
» aculeata
» odontella
Evernia furfuracea
» prunastri
Alectoria chalybeiformis
» implexa
» jubata
Ramalina farinacea
» fraxinea
» obtusata
» pollinaria
» polymorpha
» populina
» thrausta
Usnea comosa
» dasypoga h
» fulvoreagens (h)
» glabrescens (h)
» hirta h
» rugulosa h
Physcia aipolia
» caesia
» pulverulenta
» stellaris var. rosulata
Anaptychia ciliaris
Lepraria flava (h)
Crocynia membranacea

Phytagglutinin-containing species were found to total 8 (8 per cent). Their agglutination titres are shown in table 2.

TABLE 2

TITRES OF THE AGGLUTINATING SPECIES

	Titre in	
	Saline	AB-Serum
Nephroma resupinatum	1 : 8	—
Peltigera apthosa	1 : 160	1 : 400
» canina	1 : 80	1 : 160
» erumpens	1 : 1	1 : 1
» limbata	1 : 1	1 : 1
» malacea	—	1 : 1
» polydactyla	1 : 1	—
Cladonia bacilliformis	1 : 1	—

None of the species studied revealed distinct selectivity for the blood group properties studied.

DISCUSSION

The agglutination results obtained are naturally affected by how thoroughly the agglutinating factor is transferred in the filtrate. The majority of the lichen species studied could fairly easily be ground into fine powder. As all the species in which agglutination was observed, however, belonged to the difficult-to-grind group, it seems improbable that therefore the number of positive species should be notably reduced. It is of interest that the species containing agglutinins are mainly found in the genus Peltigera: 2 out of the 9 Peltigera species showed strong and 4 weak agglutination, one of the latter acting only in serum milieu. One of the 2 Nephroma species showed medium-strong, and a Cladonia species weak agglutination.

SUMMARY

The ability of 10 per cent saline extracts made from lichens to agglutinate various red cells was studied. Out of the 100 species studied, 8 per cent revealed agglutinins; however, they were not specific for blood groups ABO, MN, Pp or Le.

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AUS DEM INSTITUT FÜR GERICHTLICHE MEDIZIN DER UNIVERSITÄT WIEN,
CHEM. LABORATORIUM

ZUM SPEKTROPHOTOMETRISCHEN NACHWEIS VON FILIXSTOFFEN BEI EINER TÖDLICHEN MEDIKAMENTÖSENVERGIFTUNG

von

A. R. ALHA *, H. JANSCH und F. X. MAYER

(Am 3.5. 1955 bei der Schriftleitung eingegangen)

Der gerichtschemische Nachweis von Filixstoffen in Leichenteilen bei Todesfällen ist ein sehr schwieriges Problem. Erfahrungsgemäss gelingt dieser mit gewöhnlichen chemischen Methoden oft nicht, da es nicht möglich ist, aus dem Leichenmaterial einen charakteristischen Bestandteil, nämlich die Filixsäure, frei von Verunreinigungen zu erhalten, um die Identifizierung durch Schmelzpunktsbestimmung durchzuführen.

Im Folgenden wird ein Weg aufgezeigt, der es gestattet, in Leichenmaterial die Filixstoffe auf absorptionsspektroskopischem Wege im UV nachzuweisen. Gelegenheit dazu gab ein Todesfall einer 49 jährigen Frau im Anschluss an die Medikation mit einem Wurmmittel, bestehend aus Extraktum Filicis Maris und Oleum Chenopodii.

Zur Untersuchung gelangten der Dünn- und Dickdarminhalt, sowie eine Probe des verwendeten Medikamentes (6 g Extr. Fil. Mar. und VII Gtt. Ol. Chenop.).

Zum Vergleiche wurden Dünn- und Dickdarminhalt aus der Leiche eines durch Unglücksfall verstorbenen 19 jährigen Mannes sowie je eine Probe Extr. Filic. Mar. und Ol. Chenop., bezogen von der Anstaltsapotheke des Allgemeinen Krankenhauses in Wien, als Kontrollmaterial in der gleichen Weise untersucht.

* Als WHO-Stipendist

Von jedem Untersuchungsobjekt wurde ein Extrakt sowohl aus dem Wasserdampfdestillat als auch aus dem Destillationsrückstand bereitet und dieser spektrophotometrisch untersucht.

I. ISOLIERUNG ZUR SPEKTROPHOTOMETRISCHEN UNTERSUCHUNG

1. Behandlung der Wurmmittel. — Ein Gramm des gut durchgemischten Präparates wurde mit Wasser aufgeschwemmt und mit Wasserdampft auf 200 ml destilliert. Die Hälfte des gut durchmischten Destillates wurde mit Aether erschöpfend ausgeschüttelt. Die vereinigten Aetheranteile wurden durch Stehen über Nacht mit Natriumsulfat getrocknet, filtriert, auf dem Wasserbad bei etwa 60°C vorsichtig abgedunstet und der Rückstand über Blaugel bis zum konstanten Gewicht getrocknet.

Der Destillationsrückstand wurde ebenfalls mit Aether erschöpfend ausgeschüttelt und die Aetherauszüge wie oben behandelt.

2. Behandlung der Darminhalte. — Von den Dünndarminhalten wurden 132 ml, von den Dickdarminhalten wurden 87.5 ml sowohl des Proben- als auch des Kontrollmateriales verarbeitet. Nach dem Ansäuern mit Weinsäure wurde mit Wasserdampf auf 500 ml destilliert und das Destillat mit Petrolaether ausgeschüttelt. Der Destillationsrückstand wurde auf dem Wasserbad eingedampft, die anfallende Trockensubstanz mit Alkohol ausgezogen, das alkoholische Filtrat unter vermindertem Druck verdampft und der Rückstand in heissem Wasser gelöst. Diese Lösung wurde nun einer Wasserdampfdestillation unterzogen und 100 ml abdestilliert. Dieses Destillat wurde ebenfalls mit Aether extrahiert. Der Rückstand von der zweiten Wasserdampfdestillation wurde nun mit Aether in analoger Weise behandelt.

Zur Orientierung, welche Mengen an Extraktum Filic. Mar. und Ol. Chenop. durch Wasserdampfdestillation übergetrieben, bzw. aus dem Destillationsrückstand durch Aether extrahiert werden können, wurden die einzelnen Extrakte gewogen.

In gleicher Weise wurden auch die korrespondierenden Extrakte der Vergleichsproben aus Dünn- und Dickdarminhalt ihrem Gewichte nach bestimmt. Die Mengen waren:

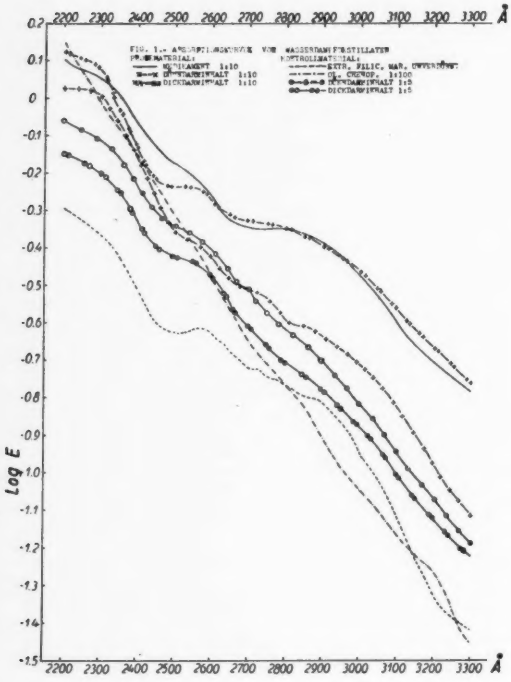
Aus den:	Extr. Filic. Mar.	Ol. Chenop.	Dünndarminhalt	Dickdarminhalt.
Wasserdampfdestillaten	3.5 mg	147 mg	4 mg	3.3 mg
Rückständen der Wasserdampfdestillation	816 mg	15 mg	1148 mg	758 mg

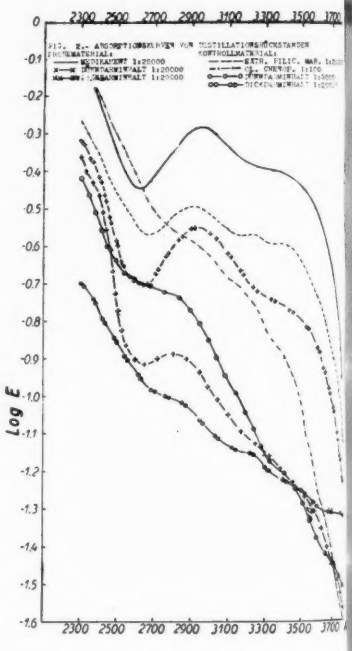
II. SPEKTROPHOTOMETRISCHE UNTERSUCHUNG

Die spektrophotometrischen Untersuchungen wurden mit den alkoholischen Lösungen der Extrakte unter Verwendung des Spektralphotometers M4Q von Zeiss bei einer Schichtdicke von 1 cm durchgeführt. Zu diesem Zwecke wurden die Aetherverdunstungsrückstände in Alkohol gelöst. Die Extrakte aus den Wasserdampfdestillaten und der Extrakt aus dem Destillationsrückstand von Ol. Chenop. wurden in je 3 ml Alkohol gelöst. Die anderen Extrakte der Destillationsrückstände wurden vor der Durchlässigkeitsmessung nach Bedarf mit Alkohol weitgehend verdünnt.

Die Untersuchungsergebnisse sind in Fig. 1 und Fig. 2 wiedergegeben. Daraus sind die angewandten Verdünnungsgrade zu entnehmen.

Aus den Absorptionskurven der einzelnen Extrakte ist zu entnehmen, dass Bestandteile von Extr. Filic. Mar. durch ihre charakteristische Lichtabsorption im UV nachgewiesen werden





können, und zwar zeigt sich, dass der Hauptanteil dieser Substanzen aus dem Destillationsrückstand isoliert werden kann. Geringe Mengen sind auch im Wasserdampfdestillat nachweisbar. Diese Substanzen zeigen eine charakteristische Lichtauslöschung zwischen 2700 und 3600 Å mit einem deutlich ausgeprägtem Maximum bei 2900 Å und einem Minimum bei 2600 Å.

Die Absorptionskurven der Extrakte von Ol. Chenop. zeigen, sowohl aus dem Wasserdampfdestillat als auch aus dem Destillationsrückstand erhalten, einen vollkommen untypischen Verlauf. Die Kurven der Kontrollextrakte aus Dünn- und Dickdarminhalt zeigen einen völlig uncharakteristischen Verlauf und entsprechen der Form, die bei solchem Material in der Regel nach dieser Aufarbeitungsart gefunden wird.

Hingegen sind in den Kurven der Destillationsrückstände der Proben, besonders aus Dickdarminhalt aber auch aus Dünndarminhalt deutlich ausgeprägte Maxima und Minima ersichtlich und zwar an den gleichen Stellen wie in den entsprechenden Kurven aus der Medikamentprobe bzw. aus Extr. Filic. Mar., der Kontrollprobe. In ihrem Verlauf sind diese Kurven weitgehend ähnlich.

Auf Grund der durchgeführten Untersuchung können folgende Schlüsse gezogen werden:

1. Die aus den Aetherextrakten sowohl der Wasserdampfdestillate als auch der Destillationsrückstände des angewendeten Medikamentes sowie aus den entsprechenden Auszügen des Extr. Filic. Mar. (Kontrollmaterial) ermittelten Absorptionskurven im UV sind identisch.

2. Die Aetherextrakte aus den Wasserdampfdestillationsrückständen des untersuchten Dünn- und Dickdarminhaltes und aus dem Extr. Filic. Mar. zeigten hinsichtlich des Kurvenverlaufes weitgehende Identität.

Erwähnt sei noch, dass die Petrolaetherauszüge jeder Hälften der Wasserdampfdestillate der Wurmmittel und der Darminhalte analog wie die Aetherextrakte verarbeitet und spektrophotometrisch untersucht wurden. Da die Ergebnisse die gleichen waren, wird darauf nicht näher eingegangen.

Die durchgeführte Untersuchung zeigt einen Weg auf, der im Gegensatz zur rein chemischen Methoden zu einem einfachen und sicheren Nachweis von Filixstoffen in Leichenmaterial führt.

Bei Ausarbeitung eines möglichst verlustfreien Isolierungs-

verfahrens wäre eine quantitative Bestimmung dieser Stoffe auch bei Anwesenheit sehr geringer Mengen im Leichenmaterial durchführbar.

ZUSAMMENFASSUNG

Die Aetherextrakte aus den Wasserdampfdestillaten und aus den Destillationsrückständen des Dünndarminhaltes und des Dickdarminhaltes einer nach Medikation mit einem Extraktum Filicis Maris enthaltenden Wurmmittel verstorbenen Frau wurden absorptionsspektrophotometrisch im UV im Vergleich mit Kontrollmaterial untersucht. Die Absorptionskurven aus den Wasserdampfdestillationsrückständen zeigten weitgehende Identität mit den Kurven, erhalten aus Extr. Filic. Mar. mit den charakteristischen Maxima bei etwa 2900 Å und Minima bei etwa 2600 Å.

FROM THE DEPARTMENT OF SEROLOGY AND BACTERIOLOGY, AND THE DEPARTMENT OF PATHOLOGY, SECTION II, UNIVERSITY OF HELSINKI

EFFECTS OF SOME ORGANIC COMPOUNDS INJECTED INTO THE SKIN OF THE RABBIT[1]

by

K. O. RENKONEN, HARALD TEIR, and KAI ENNEVAARA

(Received for publication July 15, 1955)

The following experiments were made to study the reaction of the skin of the rabbit by injecting different simple chemical compounds. The amount of the compounds was reduced to 100 γ to exclude most of the otherwise reacting compounds. Many of the compounds were so slightly water-soluble that they had to be suspended in paraffin oil. Apparently, the paraffin oil suspension did not alter the reactions because the results were the same with *e.g.*, toluquinone solved in water or suspended in paraffin oil.

The test was performed by injecting 0.1 ml of a dilution of 1: 1000 intracutaneously in the dorsum of the rabbit. Usually 10—20 tests were performed simultaneously on the same animal. The skin was previously razed with a razor blade by avoiding scratch effects as much as possible. The reaction was followed up to 48 hours. The main attention was paid to indurations, which were noted by palpation and, to some extent, by naked eye. The parts of the skin close to the spine were easiest to observe; the lateral regions, however, had to be felt with fingers.

Some 200 compounds were selected for testing. Among them were 43 alcohols, 2 mercaptans, 25 ketones, 32 acids (mainly carbolic), 9 purines, 4 sterols, 14 dye compounds and 25 quinones. Many compounds, previously known to be bacteriostatic, were included.

[1] Aided by a grant from the Sigrid Juselius Foundation.

No attention was paid to small and inconstant reactions. The noteworthiest were found in the group of quinones, which therefore was unproportionally numerous among the compounds tested. Active quinones were the following: p-toluquinone, 2.5-dimethyl-p-quinone, 2.5-dichloro-p-quinone, 2.6-dichloro-p-quinone, 1.2-naphtoquinone, and 1.4-naphtoquinone. All the other quinones involved in the experiments were significantly less active or inactive in concentrations tested. Naphta- and toluquinones gave still notable reactions in amounts of 10 γ. A very marked and persistent reaction was caused by 10 γ of toluquinone injected in the human skin.

The histological examination of the skin reaction caused by active quinones presented a considerable edema in the dermis and consequently this skin layer was 2—3 times thicker than normal. The collagen fibers were swollen and broken into fragments showing at the same time a less intense stain. A marked tissue eosinophilia was observed in the dermis where in some cases small necroses occurred. In the deepest layer of the dermis an increased number of heparinocytes was observed, especially a few days after the injection.

SUMMARY

Among 200 organic compounds injected in the skin of rabbits the group of quiuones caused most of the reactions.

FROM THE INSTITUTE OF OCCUPATIONAL HEALTH (TYÖTERVEYSLAITOS):
HELSINKI

THE FLICKER FUSION FREQUENCY IN DIFFERENT BODY POSITIONS

by

OLLI LEHTI and LEO PELTONEN

(Received for publication July 27, 1955)

The effect of several factors on the flicker fusion frequency (FFF) has already been the subject of investigation (4, 6) and reports exist on the effects of acceleration (2) and different altitudes (5), among other factors. However, no studies on the possible variations brought about by changes in the position of the body seem to have been made so far. The following observations may therefore be of some interest on this subject.

MATERIAL AND METHODS

Two healthy males aged 24 and 26 years, respectively, were the experimental subjects. The FFF was determined with the apparatus described by Fritze and Simonson (1). The flicker was exhibited for a constant length of time, which was one second. The relation between the periods of light and darkness (1 : 1) as well as the intensity of the light were also constant. Each FFF value was obtained by taking the arithmetical mean of the last readings when proceeding in, respectively, the upward and downward directions of frequency. The calibration of the apparatus was performed between determinations by comparing it with the frequency of the alternating electric current in the mains. No attention was paid to possible changes in frequency in the electricity network, as the

series of experiments were carried out during relatively short periods of time.

The FFF was determined with the experimental subject lying supine, standing upright, and held reversed with the head downwards. The supine determination was made with the subject in an exactly horizontal position, after lying as relaxed as possible for half an hour to stabilise the heart beat. The mean value of five determinations was employed. The subject then stood up and eight consecutive determinations were made during a period from zero to fifteen minutes. Since the spread was insignificant, the mean value of also these readings was employed. In the third stage of the experiment the subject was lashed to a frame by the trunk and legs and held in a vertical position with head downwards. Five FFF values were determined during zero to five minutes. Also here the spread was small and the mean value is therefore employed.

In all three positions the distance between the eyes and the source of flickering light was maintained constant. Similarly the background was kept unchanged. The subject focused directly on the source of light. — The experiments were performed on both subjects in all three positions on five different days at the same hour.

RESULTS

In order to eliminate daily variations, the mean supine value for each day is designated as 100. The daily mean values for the other positions are then expressed as relative values. This makes it possible to combine the results for the two experimental subjects and thus we have ten series of determinations from which the final mean values are calculated for each position.

The mean value with the subject standing upright was 99.6, which does not significantly differ from the supine value of 100. In the head down position the mean value was 102.13 (standard deviation: 2.24; mean error: 0.707), which is a statistically significant increase (in the t-test, $P < 0.01$). In absolute figures the increase was from 43.4 (mean value in the supine position) to 44.4 (mean value in head down position).

DISCUSSION

Since the FFF depends at least to some extent on the functioning of the retina (4), the elevated value in the head down position might be ascribable to an improved circulation of blood in the retina. However, this opinion is contradicted by the observation that there is no correlation between the retinal circulation and the FFF (3). The level of FFF has also been held to be an indicator of the state of excitation of the central nervous system (4). This might also provide an explanation for the elevated FFF values obtained in the head down position. The brain would here be stimulated by an increased amount of blood and an increased cerebral pressure. On the other hand, since the FFF value has been found to be considerably decreased in anaemia and anoxia (5, 3) it would appear probable that hyperaemia and an increased partial pressure of oxygen have an opposite effect. The rise in the FFF would thus find its explanation on a purely circulatory basis.

SUMMARY

The flicker fusion frequency was studied in two young male subjects when lying supine, standing upright, and held inverted with the head downwards. Five series of determinations were made on each subject. The values when standing did not significantly differ from the supine values. With the subject in the head down position there was a significant increase as compared with the supine values (mean values = 44.3 and 43.4, respectively; $P < 0.01$).

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FROM THE DEPARTMENT OF SEROLOGY AND BACTERIOLOGY, UNIVERSITY OF HELSINKI

BACTERIA FOUND IN THE EPITHELIAL CELLS OF HUMAN LIPS

by

TIMO KOSUNEN

(Received for publication September 12, 1955)

On the basis of descriptions given by several investigators Harkness has made a summary of the bacteria which are encountered in the human urethra and the urethral epithelial cells (1).

The present study deals with the bacteria found in the epithelial cells of human lips as well as with the age of their first manifestation. The microphotographs taken for this study are almost identical with those taken by Harkness from the epithelial cells of the human urethra in desquamative urethritis (1) as well as with some photographs of Rickettsiae published by Donatien and Lestoquard (2), e.g. from the epithelial cells of the sheep conjunctiva.

The smears were taken from sixty medical students and laboratory workers as well as from seventy-six newborn infants at the Helsinki University Women's Clinic. A sterile and clear slide was pressed against the lower lip, from which the distal epithelial cells stick to the glass. The slides were fixed in a Bunsen flame, stained for two minutes in a 1 per cent neutral red solution, to which 0.2 ml of 1 per cent acetic acid was added per 100 ml. The excessive dye was washed off and the preparates were dried between blotting-papers.

RESULTS

The staining method used makes the protoplasm of cells appear pink, whereas the nuclei and the bacteria in the cells become darker

red in colour. The majority of the bacteria found were cocci, diplococci, and short rods; streptococci, long rods and rods in chains were found less frequently. There are 2—3 kinds of these bacteria in one cell, and the different cells of the same preparate are similar in their bacteria content. As a rule, the variability is greater with adults than with newborn infants, who very often had cells containing one form of bacteria only. The amount of bacteria varies from a few to innumerable bacteria, in which the whole cell is packed with bacteria. The frequency of positive findings is shown in Table 1.

TABLE 1

Age	Positive	Negative	Total	Percentage of Positive Findings
0—12h	0	38	38	0
13—24h	7	31	38	18
25—48h	65	11	76	86
49—72h	74	2	76	97
73—96h	76	0	76	100
Adults	60	0	60	100

The newborns are taken to their mothers' breast for the first time at the age of 24 hours or at the same age as the bacteria usually appear in the epithelial cells of human lips. Infants delivered by Cesarean section have, however, their first breast-feeding not until the age of about three days, and five of nine of these infants showed negative findings still at the age of 2½ days. If we separate the infants delivered by section from the above Table the figures will change as follows:

TABLE 2

Age	Positive/Total		Percentage of Positive Findings	
	Normal Delivery	Section	Normal Delivery	Section
0—12h	0/33	0/9	0	0
13—24h	6/34	1/9	18	11
25—48h	61/67	4/9	91	44
49—72h	67/67	7/9	100	78
73—96h	67/67	9/9	100	100

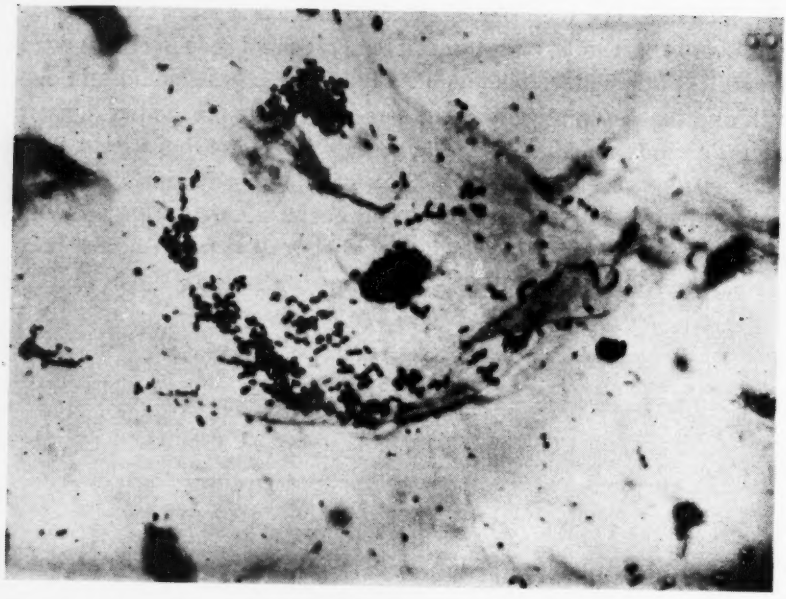

Fig. 1. — Epithelial cells with epithelial bacteria taken from the lip of a 2-day-old infant. Magnification 750.

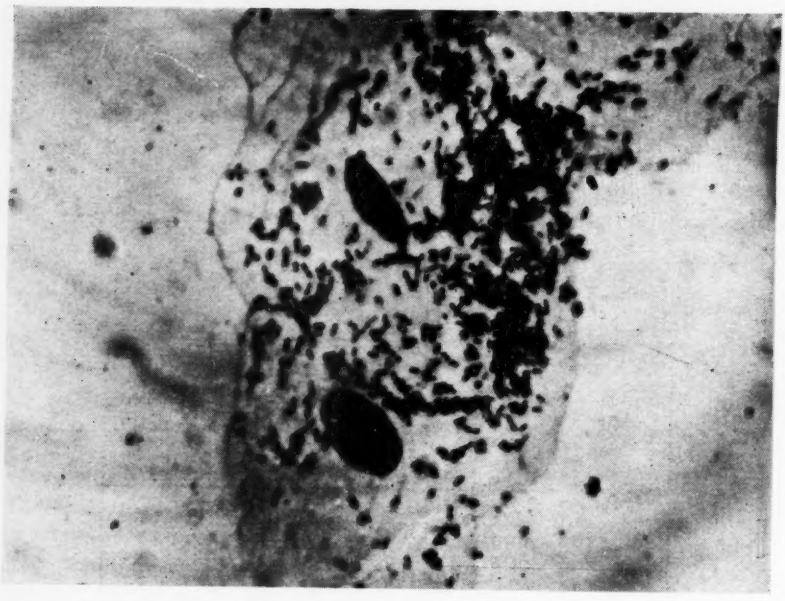

Fig. 2. — Epithelial cells with epithelial bacteria taken from the lip of an adult. Magnification 750.

It would seem as though the bacteria would appear later in the epithelial cells of the newborns when there is a delay in the breast-feeding, and when an eventual contamination from the vaginal epithelium is avoided at birth.

CONCLUSIONS

The epithelial cells of the lips of adults contain regularly epithelial bacteria. The manifestation of epithelial bacteria simultaneously with the first breast-feeding in infants delivered by section would indicate that the bacteria originate in the skin of the mother's breast. Indeed, there is also the possibility that the children delivered in the normal way get contaminated from the vaginal epithelium at birth.

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FROM THE DEPARTMENT OF ANATOMY, UNIVERSITY OF HELSINKI

EFFECT OF THYROID EXTRACT ON THE SEMINAL VESICLE OF CASTRATED RAT[1]

by

ANTTI TELKKÄ, MATTI TUOVINEN and A. N. KUUSISTO

(Received for publication September 26, 1955)

Recently we reported that the administration of thyroid extract to intact rats seemed to have a stimulating effect on the seminal vesicle (5). The present paper deals with a continuation of this experiment, in which we tried to find out whether the administration of thyroid extract affects the seminal vesicle of a castrated rat as estimated from the weight of this gland and from the width/height ratio of its epithelial cells.

MATERIAL AND METHODS

Thirty albino rats of the Wistar strain, weighing about 150 g, were used. Ten rats served as intact controls. Twenty rats were castrated under slight aether anaesthesia 30 days before sacrifice. During the last 10 days of the experimental period ten of them received 0.4 ml of thyroid extract daily (»Thyranon», Organon, corresponding reportedly to 0.04 mg of organically bound iodine) in subcutaneous injections. The animals were sacrificed by decapitation and bled. The thyroid, pituitary, adrenal and thymus glands and the seminal vesicles were removed and weighed. The thyroids and the seminal vesicles were fixed in Bouin's fluid and stained with Mallory's azan method. For the determination of the percental proportion of the thyroid epithelium, colloid and stroma, the method

[1] Aided by a grant from the Sigrid Jusélius Foundation

of Uotila and Kannas (6) was followed. The width/height ratio of the epithelial cells of the seminal vesicle was measured from a projected picture of the specimen and expressed as a mean from measurements of 100 cells.

RESULTS

The mean initial and final body weights, the absolute and relative weights of the seminal vesicles, and the width/height ratio of the epithelial cells of the seminal vesicle are given in Table 1.

TABLE 1.

	Intact Controls ($n = 10$)	Castration ($n = 10$)	Castration plus Thyroid Extract ($n = 10$)
Initial body weight (g)	164± 9	163±8	163±11
Final body weight (g)	181±10	188±9	173±11
Weight of seminal vesicles (mg)	650±19	102±7	123±12
Weight of seminal vesicles (mg/g of body weight)	3.59± 0.6	0.55±0.04	0.76± 0.06
Ratio width/height of the epithelial cells of the seminal vesicles	33.4 ± 0.9	79.7±0.7	57.9 ± 0.6

The weights of the seminal vesicles and the width/height ratio of the intact controls differed significantly from those of the two castrated groups. When comparing the absolute and relative weights of the seminal vesicles in the two castrated groups it was found that the weights tended to be higher in the thyroid extract-treated group, but this difference is statistically probably only. In contrast, the width/height ratio is significantly greater in the castrated group, *i.e.* the epithelial cells of the seminal vesicle are more flattened and nearly cuboidal.

There were no significant differences in the mean adrenal and pituitary weights between the three groups. The mean weight of thymus was below that of the controls in the thyroid-extract treated group and above that of the controls in the castrated group.

The weights of the thyroid and the percental proportion of the thyroid epithelium, colloid and stroma were as follows:

	Mean Weight of the Thyroid (mg)	Epithelium	Colloid	Stroma
Intact Controls	20.0±1.2	77.0±1.6	15.4±1.0	7.6±0.2
Castration.....	19.8±1.3	74.7±2.0	15.2±0.9	10.1±0.2
Castration plus Thyroid Extract	13.3±1.5	46.1±2.2	34.1±1.6	19.8±1.0

As could be expected, was the mean weight of the thyroid in the thyroid extract-treated group significantly lower than those of the two other groups. The low epithelium and increased amount of colloid in this group indicates a thyroid inactive as far as the histological picture is concerned. No differences could be established between the values of the two other groups.

DISCUSSION

It has been claimed in earlier works (2, 3) that castration results in an inactivation of the rat thyroid. We were not able to observe any inactivation, and also according to the prevalent concept castration generally remains without any striking effect upon the thyroid (4)

According to our results, the treatment of castrated rats with thyroid extract seemed to have a stimulating effect on the epithelium of the seminal vesicle. The shape of the epithelial cells became more columnar, a change which could be expressed with the width/height ratio used. The changes in the weight of the seminal vesicles were not so marked.

When discussing on the mechanism of this stimulating effect in our previous report (5), primarily three possibilities were mentioned: the stimulating effect of thyroid extract is mediated through the anterior pituitary; the stimulation is connected with the non-specific action of thyroid hormone on the metabolism of all the body cells; or there is direct relationship between the thyroid and gonads. In the light of the present observations the last-mentioned possibility seems improbable. The stimulative effect is possibly connected with the non-specific action of thyroid hormone, or it is mediated through the pituitary. In this connection the finding of Eartly and

Leblond (1) must be mentioned. They observed that in thyroidectomized rats, thyroxine promotes the growth of gonads only in the presence of the hypophysis.

SUMMARY

The effect of thyroid extract on the weight and on the width/height ratio of the epithelial cells of the seminal vesicles of castrated rat has been investigated. The administration of thyroid extract seemed to have a stimulating effect on the seminal vesicle of castrated animal, too. The causes of this effect are discussed.

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FROM THE DEPARTMENT OF PHYSIOLOGY, UNIVERSITY OF HELSINKI

CHOLERESIS INDUCED BY CONTINUOUS INTRAVENOUS INFUSION OF SODIUM DESOXYCHOLATE UNDER HYPOXIA

by

EEVA JALAVISTO [1], E. AANTAA & SEIJA AANTAA, and R. ELOSUO

(Received for publication September 27, 1955)

The secretion of bile and bile pigments is not very sensitive to hypoxaemia, as experiments of Tanturi and Ivy (10) on narcotized dogs under short periods of hypoxia and those of McLachlan, Sleeth and Gower (7), on prolonged exposure to low pressure of conscious rats with biliary fistulae have shown. (Further references may be found in Hanzon (5).) However, in extreme anoxia (3—5 per cent O_2 in the inspiratory air) the secretion always decreases which seems to indicate that it is not question of an anaerobic process (3, 4, 8). Previous experiments (6) on the excretion of intravenously injected bilirubin under hypoxia in conscious and in narcotized dogs showed that it was not possible to obtain a retention of the dye of any significance even in highest tolerated hypoxia corresponding to an oxygen concentration of 6.5 per cent in inspired air. This might be explained by assuming that the elimination of bilirubin from the blood and its secretion into the bile are two processes, which are differently affected by the hypoxaemic condition. The experiments of Wirts and Cantarow (12), Brauer and Pesotti (1), are in conformity with this view. The fluorescense-microscopic picture of uranin secretion under hypoxia gives further support to this assumption. During hypoxia narcotized rats showed accumulation of

[1] Aided by a grant from the Sigrid Jusélius Foundation.

uranin within the liver cells at the same time that the biliary capillaries remained empty (5).

The studies concerning different functions of liver in anoxia are complicated first of all because the regulatory changes of the circulation within the liver are not entirely under experimental control. A second complication is that the liver cells lie at varying distances from the O_2-supplying blood vessels. Therefore, e.g., the function of cells lying in the proximity of the central veins may be impaired at blood oxygen levels which do not in the least affect the more periferally situated cells. If the secretory capacity of liver cells for some substance is limited so that (as for bilirubin and uranin) an increased concentration in blood leads to an increased secretion only until the »ceiling» for secretion is attained, a decrease in the number of functioning cells must lead to a diminished secretion of such a substance. On the other hand if such limiting secretory »ceiling» does not exist under reasonable conditions the reduction in functioning cells would be without effect except under extreme anoxia since a few functioning cells would fully compensate the failure of others. According to Hanzon the liver cells show a »secretory ceiling» for bilirubin, but not for cholates. If a decreased secretion of cholates can nevertheless be demonstrated under hypoxic conditions only three possibilities for explanation seem to exist: 1) all cells are affected 2) hypoxia provokes a (or lowers the) secretory ceiling for cholates. 3) The concentration of cholates in blood remains low through absorption of cholates by non-secreting cells, which prevents the functioning cells from increasing their secretion to a level high enough to compensate for the loss of function in others.

The aim of the investigation reported in the present paper was to throw light on the hypoxic secretion of cholates.

MATERIAL AND METHODS

Preliminary experiments were carried out on cats, and sodium desoxycholate was administered as single intravenous injections. It soon became clear that single injections were not well tolerated and the secretory capacity of a cat's liver too small in relation to a drop recording system (the number of drops per minute in normal conditions was only about 1). Consequently the changes induced by

the injection and of hypoxia were irregular. The main experiments were therefore performed with 7 dogs, which were anaesthetized with nembutal or in the three last dogs with intravenous administration of thio-pentone, approximately 0,015/kg, supplemented when necessary with additional amounts during the course of an experiment. The dogs were heparinized in order to secure continuous blood pressure recording from a canulated carotid artery. The cystic duct was ligated and the common bile duct canulated with a thin polyethylen tubing (bore diameter = 1 mm). The bile flow was registered with a drop recorder described by Wesson (11) which allows simultaneous volymetric measurement of the bile flow. This was done by letting the drops collect in a calibrated small byrette provided with a stopcock for emptying the byrette and marks at 0.1 and 0.2 ml. The time required for accumulation of 0.2 ml bile was taken at times with a stopwatch. The drop receiver byrette was rinsed with a drop of octanol in order to prevent foaming and adhesion of the drops on the walls. For constant infusion of desoxycholate a polyethylene tubing was tied in the femoral vein. The dog was placed in a body pletysmograph for registration of the respiration. The tracheal canula was connected to a pair of respiratory valves and the hypoxia was effected by leading a stream of a low concentration oxygen nitrogen mixture in the inspiratory valve. An oxygen percentage of 4.6—5 per cent is tolerated only during a few minutes during which the bile flow is much reduced or stops altogether both in cats and dogs. These concentrations are therefore not suitable for longer experiments. An oxygen concentration of 6 and 6.5 per cent is already fairly well tolerated. The range of oxygen mixtures was therefore 6—9 per cent O_2 with 94—91 per cent nitrogen. The gas was delivered from gas containers through a large rubber tubing inside which the inlet tube of the inspiratory valve was put so that there was no risk for admixture with atmospheric air. The periods of hypoxia varied from 15 minute to 1 h. 30 minutes.

Na-desoxycholate for infusion was prepared by making first a 2 per cent stock solution by diluting desoxycholic acid in 0.5 per cent Na_2CO_3. This stock solution was then diluted with physiologic saline to form either 0.4 or 0.8 per cent solutions. All solutions were made of pyrogenfree material. The constant speed infusion pump was adjusted to deliver 1 ml/min. (0.9—1.05).

RESULTS

A typical record of the effect of hypoxia on spontaneous flow of bile is seen in fig. 1. Fig. 2 represents a similar experiment with infusion of desoxycholate. The sensitivity of bile flow to hypoxia varies so much in different animals, that no definite limits of

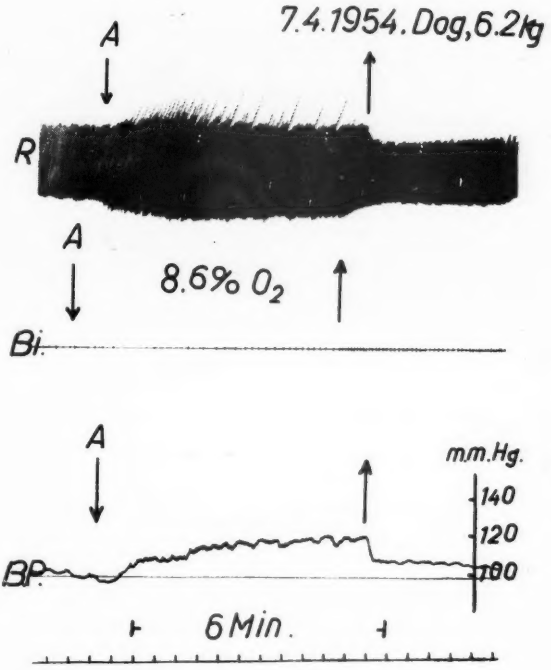

Fig. 1. — Spontaneous flow of bile. Between arrows (left to right) breathing of 8.6 per cent O_2 in N_2. R = respiration, Bi = bile drop record. B.P. = blood pressure. Time 30 seconds.

tolerance can be established. As may be seen from Fig. 1 and 2 8.6 and 8 per cent O_2, may decrease both the spontaneous bile flow and that stimulated by infusion of desoxycholate. On the other hand in an other dog (Fig. 3) 7 per cent O_2 does not at all interfere with bile flow. Whether these differences are due to differences in the general condition of the animal on the depth of anaesthesia is not possible to asess. The latter possibility seems most probable since e.g. sodium thiopentone clearly depresses the bile flow although the effect is of short duration, as is seen from fig. 3.

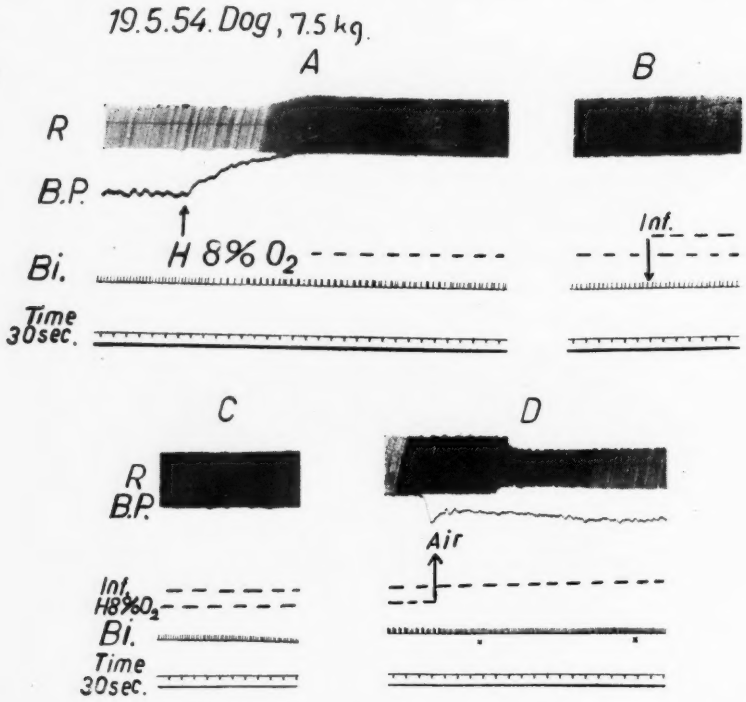

Fig. 2. — Infusion of sodium desoxycholate, 0.4 per cent solution, 0.9 ml/min. during hypoxia (8 per cent O_2 in N_2). A. Introduction of hypoxia at H. B. 12 minutes after A. Infusion started at arrow 28 minutes after beginning of hypoxia at H. C. 10 minutes after B. D. 15 minutes after C when the hypoxia has lasted for 52 minutes and infusion of Na-desoxycholate for 34 minutes. At arrow breathing of air restored, infusion continues.

The depressing effect of hypoxia on bile flow is initially greatest. The rate of flow is later entirely restored or remains on a lower level. After changing to breathing of air an acceleration of bile flow is usually seen also in those cases in which the rate of flow has apparently been restored as e.g. in fig. 1. The rate of flow during the last minute before hypoxia is 7 drops/min. during the last minute of hypoxia (8 per cent O_2) likewise 7 drops/min. but during the first minute after discontinuation of hypoxia 13 drops/minute. Obviously the same rate of flow can under hypoxic conditions be maintained but the retention of bile which has occurred during the induction of hypoxia cannot be eliminated. The question of a secretory ceiling therefore arises. Experiments with continuous infusion of desoxy-

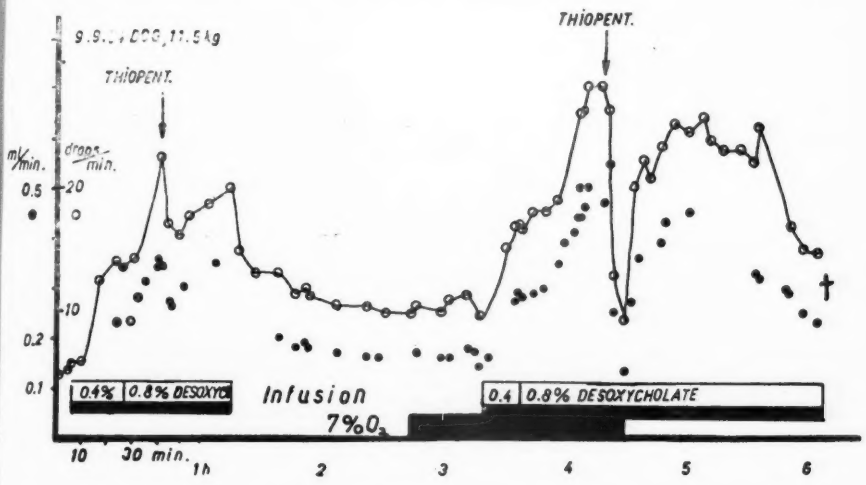

Fig. 3. — Graph showing the effect of infusion of sodium desoxycholate on rate of drops and volume flow of bile. Black and white: infusion. Black: hypoxia, 7 per cent O_2. At arrows i.v. injections of thiopentone (2.5 and 2.0 ml, 0.5 per cent solution). Circles: drops per minute, solid circles: volume flow ml/min.

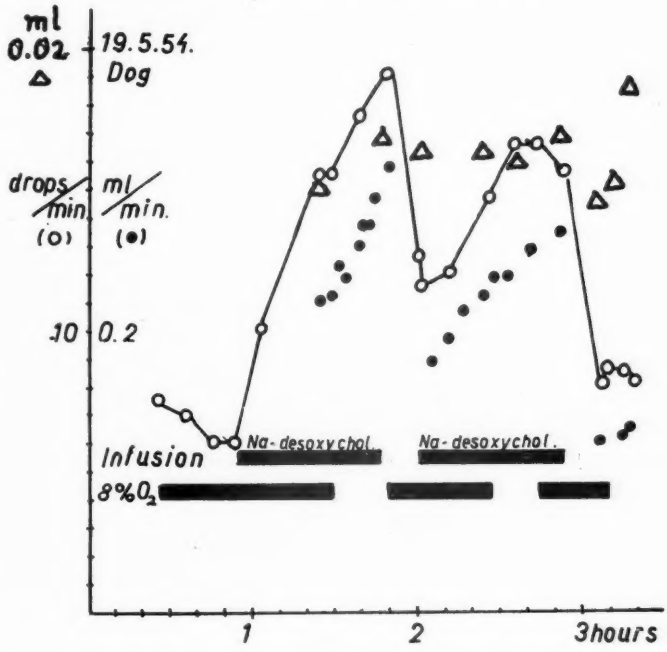

Fig. 4. — Infusion of Na-desoxycholate after introduction of hypoxia (8 per cent O_2). Circles: drops per minute. Solid circles: volume flow, ml/min. Triangles: drop volume, ml.

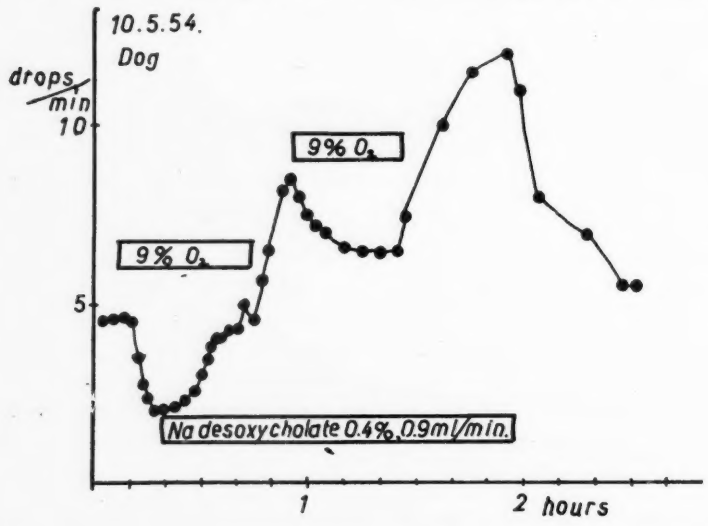

Fig. 5. — Graph showing the effect of infusion of Na-desoxycholate started during av period of hypoxia (9 per cent O_2), and that of hypoxia during infusion, on rate of drops.

cholate during different degrees of hypoxia show without any doubt that the depressed rate of bile flow during hypoxia does not represent the secretory ceiling. As seen from figure 3, 4 and 5 which represent experiments with 7, 8 and 9 per cent oxygen in inspired air, infusion of Na-desoxycholate increases the rate of bile flow many times over the initial level during the hypoxic period. In the experiment with 7 per cent oxygen (fig. 3) which does not cause any depression of bile flow the rate of flow stimulated with 0.4 per cent sodium desoxycholate actually increases the flow beyond that reached in the control experiment during a corresponding time period. On the other hand if during the hypoxic period a depression of the spontaneous flow has been noted the introduction of hypoxia during the infusion leads likewise to a marked diminution of the output of bile (Fig. 5 and 6). The flow of bile settles down to a fairly constant level which is the higher the longer the infusion has lasted before introduction of hypoxia.

In order to get some idea of whether the concentration in bile of cholates varies much through the experimental procedures the average drop volume was calculated by dividing the volume flow with the number of drops. An increased drop volume would mean

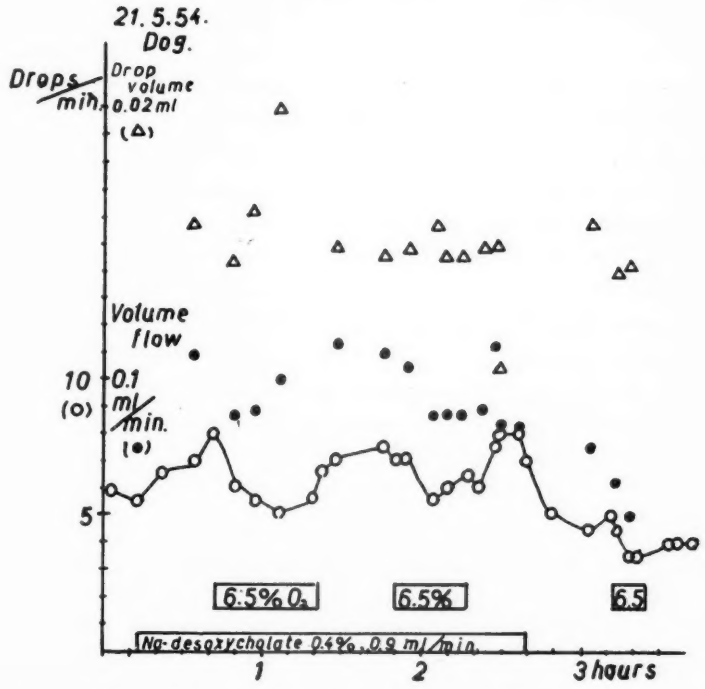

Fig. 6. — Graph showing the effect of hypoxic periods (6.5 per cent O_2) on rate of drops, volume flow and drop volume during continuous infusion of Na-desoxycholate.

increased concentration of cholates. No clearcut evidence of regular variations were obtained. An example of the fair constancy of drop volume may be seen in fig. 4 and 6 (triangles). A transient diminution of drop volume during anoxia seem to be more common than the opposite, but the method is not accurate enough for definite conclusions.

The infusion of sodium desoxycholate is well tolerated during many hours, and without effect on the blood pressure if the concentration does not exceed 0.4 per cent at a rate of 1 ml/min. This concentration seems, however, be close to the limit of safety .0.8 per cent solution leads within 40 minutes to a very marked intravascular haemolysis and depression of the blood pressure. Even with the smaller dose hematuria develops but the elimination of hemoglobin in the urine keeps nearly pace with the haemolysis: blood samples are only moderately haemolytic.

DISCUSSION

Since a chemical method of sufficient accuracy for quantitative determination both of the output of cholate in bile and the concentration of cholate in blood, was not at our disposal only indirect evidence for the mechanism of the hypoxic depression of bile flow can be gained from the experiments related above.

Three facts are to be considered. 1) Well tolerated hypoxaemic conditions, if sufficiently severe, lead to depression both of the spontaneous flow of bile and that stimulated by a constant speed infusion of cholate. 2) The secretory capacity under these circumstances is not solely determined by the severity of anoxia but primarily by the supply of choleretic substances. 3) During hypoxia a secretory »ceiling» manifests itself if the hypoxia is severe enough to cause a depression of the spontaneous flow of bile; if the spontaneous output is not affected the maximum capacity of bile secretion is not less than during breathing of air.

Obviously the liver cells cannot be all affected uniformely, nor does the number of functioning cells be the factor which determines the level of bile flow under hypoxia. Since the limit of the secretory capacity varies with the amount of cholate accumulated during the infusion the most probable explanation would be that there are two interfering factors: elimination of the cholates from the circulation by nonfunctioning cells which opposes the stimulating effect of increasing concentration of cholates in blood. The apparent lack of a definite »ceiling» for cholate under normal conditions and the appearence of such a limit during hypoxia is surely only a difference of degree and depends most likely on the amount of functioning tissue differing in these two conditions. Thus as supposed already by Hanzon any principal difference between the secretion of bilirubin, uranin and cholates by the liver cells does probably not exist.

Although not particularly studied, the extremely striking inhibition of bile secretion by injections of thiopentone, is interesting. Thiopentone belongs as well known to the relatively short acting barbiturates and is mainly detoxicated in the liver. The barbiturates are probably not secreted into the bile, since they do not appear in faeces (9). That substances like bilirubin and uranin and to some extent bromsulphalein compete for the secretory

mechanism of liver cells (2, 5), is known, but whether detoxication mechanisms likewise interfere with the secretory ones has not been elucidated. In case of narcotics a general depressing effect on all aerobic cell functions is a further possibility, but then the different narcotics should likewise have a similar depressing effects on bile flow. Since this question fell out of the scope of this study no systematic evidence can be obtained. The general impression was that e.g. intravenous injections of nembutal did not interfere with the bile flow as did thiopentone. This might, of course, also be a manifestation of the well known toxic effect of thiopentone on liver cells.

SUMMARY

1. The effect of hypoxia produced by inhalation of 6—9 per cent oxygen in nitrogen on the spontaneous bile flow and that resulting from stimulation by continuous infusion of sodium desoxycholate, was studied in 7 dogs.

2. The sensitivity against hypoxia varied in different preparations: a 7 per cent O_2 mixture sometimes was without effect whereas a 9 per cent mixture had a clearly depressing effect both on spontaneous flow and that stimulated by cholate.

3. The depressed bile secretion under hypoxia could always be increased by stimulation with continuous infusion of 0.4—0.8 per cent sodium desoxycholate at a rate of 1 ml/min. The rate of bile flow attained thereby values considerably above the initial level.

4. A secretory »ceiling» was demonstrable during hypoxia. Its level was not solely determined by the degree of anoxia, but primarily by the amount of cholate fed to the liver cells.

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FROM THE MUNICIPAL BACTERIOLOGICAL LABORATORY, HELSINKI, AND THE DEPARTMENT OF VIROLOGY, UNIVERSITY OF HELSINKI

GROWTH OF THE EGG-ADAPTED ROUS SARCOMA IN RELATION TO THE AGE OF THE EMBRYO[1]

by

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Rous and Murphy adapted the Rous sarcoma to embryonated chicken eggs as early as 1911 (21). Since then several publications have appeared on the subject and much has been learned about the behaviour of the Rous sarcoma in embryonated eggs (5, 12, 13, 17, 21). An observation of great interest was that by Milford and Duran-Reynals of the hemorrhagic disease in the embryo caused by the Rous sarcoma (14).

In a previous study on the behaviour of the virus of chicken and duck tumours in the embryonated egg of chickens and ducks (17) the intention was to study this hemorrhagic disease among others. The amount of virus present in the tumours grown on the chorio-allantoic membrane was compared with the amount of virus in the embryo at different intervals after the inoculation of the eggs. In this work, 8 to 10 day embryos were usually used. A brief survey of the literature in this field shows that embryos between 6 and 15 days of age have been used, most usually probably 8-day embryos (5, 12, 13, 14, 17, 21).

In attempts to transplant human tumours to the chorio-allantois of embryonated eggs it was assumed that a long incubation period would favour the growth of the tumour transplants, and in some experiments even 7-day embryos were used. Some times 11-day

[1] This investigation was aided by grants from Sigrid Juselius' Stiftelse.

embryos were also used, but on an average the embryos were 9 to 10 days old (18). The prolonged incubation period did not, however, in our opinion, result in any better growth of the transplants.

As was the case with the work on the Rous sarcoma, there seems to be a fairly wide variation in the age of the embryos used for transplanting heterologous tissue to the chorio-allantois of embryonated chicken eggs (3, 4, 8, 9, 10, 11, 12, 13, 20, 23, 24, 25). An investigation concerning this problem has recently been performed by Alfthan (1).

In this country, Penttinen (19) has also paid attention to the age of the embryo when cultivating vaccinia virus on the chorio-allantois. Most of the literature concerning the role of the age of the embryo in relation to virus infection has been reviewed by Beveridge and Burnet (2), and more recently by Sigel (22). The age of the embryo is thus of great importance for virus cultivations in the embryonated egg. It therefore seemed that an investigation of the role of the age of the embryo in tumour growth might be of some interest. This paper consequently deals with some experiments on the growth of the egg-adapted Rous sarcoma in embryonated chicken eggs incubated for varying lengths of time before inoculation of the tumour suspension in the chorio-allantoic membrane.

MATERIALS AND METHODS

The Rous sarcoma was obtained from Dr. F. Duran-Reynals, Yale University, New Haven, Conn., U.S.A. in 1950 and has since then been kept in this laboratory in alternate chicken and egg passages.

The routine passages in eggs were made by inoculating about 0.05 ml of a 20 per cent tumour suspension into the chorio-allantoic membrane of 5 to 10 eggs incubated for 8 to 12 days at 37°C before inoculation.

The tumour suspension was prepared in a phosphate buffer, pH 7.2, containing 50 units of penicillin and 50 μg of streptomycin per ml, and in all other respects as reported in a previous work (17). The inoculation of the membrane was performed by the technic described by Beveridge and Burnet (2). The window in the shell was sealed with scotch tape.

For the experiment proper eggs incubated at 37°C for 7, 8, 9, 10, 11, 12 and 13 days respectively were inoculated on the same day in to the chorio-allantoic membrane with 0.05 ml of a 20 per cent tumour suspension of sarcomas grown in the chorioallantois of 8-day embryos for 10 days. Altogether 252 eggs were inoculated. The eggs were incubated further at 37°C and every second day a number of eggs (two to ten) from each group were opened and the tumours or the membranes harvested. The membranes and/or the tumours from each group on each second day were pooled and weighed. The mean weight of the tumours was calculated.

One membrane or tumour was taken from each pool for sectioning.

Each embryo was dissected and examined macroscopically for hemorrhagic lesions.

Tissues sectioned were fixed in 10 per cent formalin, cut at 10 microns and stained with hematoxylin and van Giesons stain.

RESULTS

Throughout the work it was apparent that there was a fairly great variation in tumour size even in embryos of the same age. One reason for this was probably the difficulty of exposing the membrane in exactly the same way at inoculation, a point which was made in the earlier paper (17). Taking the mean weight of the tumours, however, it was evident that there was a clear difference in tumour size that depended on the age of the embryo at the moment of inoculation (Table 1).

The largest tumours were obtained in 11-day embryos and the smallest in 7- and 13-day embryos. Between the final size of the tumours from embryos 8, 9, 10 and 12 days old there was no great difference.

Comparing the time of incubation with tumour size showed that fairly big tumours were obtained from the embryos of 10, 11, 12 and 13 days within 6 days of incubation. After this period of incubation the membranes of the 7, 8 and 9 days old embryos showed only very small tumours or no tumours at all, and tumours of the same size as in the embryos of 10, 12 and 13 days old were not obtained until 2 days later (Figure 1).

From a comparison of tumour size with embryo age independent

TABLE 1

MEAN WEIGHT OF TUMOURS ON THE CHORIO-ALLANTOIC MEMBRANE AND INCIDENCE OF HEMORRHAGIC LESIONS IN EMBRYOS INOCULATED WITH A 20 PER CENT CELL SUSPENSION OF THE ROUS SARCOMA.

Age of Embryo at Inoculation	Number of Eggs Inoculated	Number of Eggs Discarded	Time in Days after Inoculation																	
			2			4			6			8			9			10		
			No [3]	TMW [4]	HL [5]	No	TMW	HL	No	TMW	HL	No	TMW	HL	No	TMW	HL	No	TMW	HL
7 days	36 [1]	9	6	142	0	6	175	0	6	242	0	4	543	4				5	1371	1
	11 [2]	0				3	166		2	243					3	1407		3	1137	
8 days	37	14	6	229	0	3	328	0	5	492	0	5	1220	5				4	1560	4
	15	0				3	293		2	342					5	2682		5	2566	
9 days	36	9	6	200	0	6	364	0	4	474	0	5	2392	4				5	1670	1
	14	0				3	379		2	566					3	3031		6	2897	
10 days	35	10	6	195	0	5	378	0	4	1316	4	5	1762	5						
11 days	30	7	5	255	0	5	670	0	5	3229	4	8	4530	8						
12 days	28	5	6	360	0	6	733	0	6	1596	1	5	2202	2						
13 days	29	9	5	172	0	9	597	0	6	1483	0									

[1] First experiment
[2] control experiment
[3] number of eggs tested
[4] tumour mean weight
[5] number of embryos showing hemorrhagic lesions

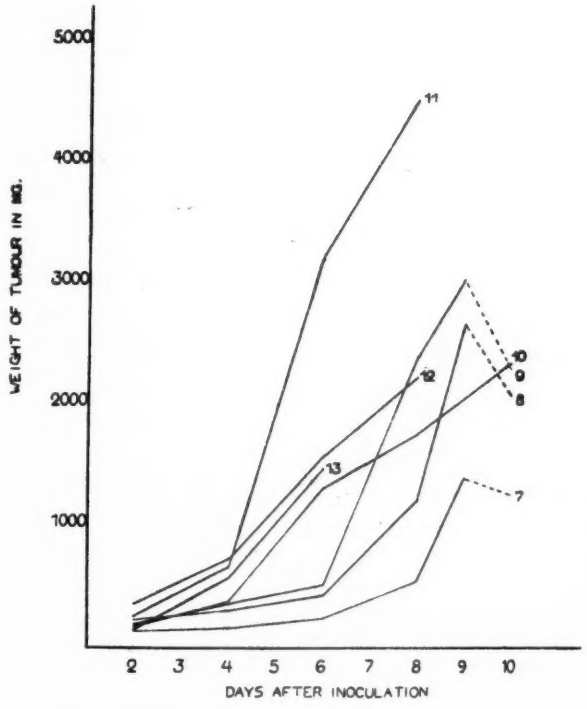

Fig. 1. — Mean weight of tumours on the chorio-allantoic membrane of embryonated eggs of different ages. Tumour weight is correlated to time of incubation after inoculation. The numbers for each line indicate the age of the embryo at the time of inoculation.

of the age of the embryo at the time of inoculation, it was seen that the actual increase in tumour size began when the embryo was 14 to 16 days old and reached its maximum 3 to 6 days later (Figure 2). In embryos of 7, 8 and 9 days there did not, therefore, seem to be any actual tumour growth during the first days. In the 7-day embryos the tumours never, whatever the prolonged incubation period, reached the same size as the tumours in the other embryos, with the exception of the 13-day embryos for which the incubation period was too short.

The 11-day embryos differed from all the other embryos tested in that the size of the tumours, in this particular experiment, was far greater than that of the others at any interval tested.

There was no further increase in tumour size in the embryos

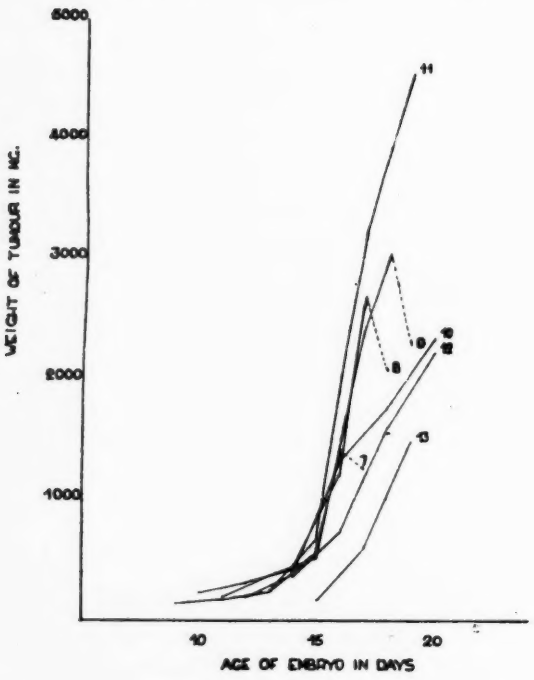

Fig. 2. — Mean weight of tumours on the chorio-allantoic membrane of embryonated eggs of different ages in correlation to the age of the embryo at the time when tumours were harvested. The numbers for each line indicate the age of the embryo at the time of inoculation.

of 7, 8 and 9 days after 9 days of incubation; on the contrary, there was rather a decrease in the size of the tumours.

There was a fairly close correlation between the incidence of hemorrhagic lesions in the embryo and the size of the tumours (Table 1). The same was true of the relationship between hemorrhagic lesions and the death of the embryos, if we exclude the embryos which died very early during the experiment.

In the microscopic picture of the tumours in the different groups no differences could be observed.

After the completion of this series the correlation between the size of the tumours and the age of the embryo at inoculation was followed in every routine passage for a period of about one year. Altogether 356 embryonated eggs of different ages were used for

31 passages. The mean weight of the tumours from these passages usually corresponded well to the results obtained in the above experiment.

During this time, however, additional observations were made which are briefly mentioned here. It was noticed that if a tumour grown in an 8-day embryo was transferred to 11-day embryos the usual result was far bigger tumours than in the previous 8-day passage. This was true also of tumours grown in 10-day embryos and transferred to 11-day embryos. Sometimes, however, the opposite was seen, and the transplantation of tumours from 8-day embryos into 11-day embryos resulted in still smaller tumours. These, however, after serial passages in 11-day embryos, increased in size, and after several passages reached the same size as the usual 11-day tumours. A similar state of affairs was seen when the tumour tissue had been stored in the ice box for some length of time. Serial passages however usually restored the tumours very fast. The longest period of storage tested was five months, after which time tumour growth still resulted on the chorio-allantoic membrane.

DISCUSSION

The influence of the age of the host on virus infections has recently been reviewed by Sigel (22) and much of the literature concerning the age of the chicken embryo in relation to virus infections has been cited in this survey. As far as the tumours, and especially the Rous sarcoma, are concerned, Duran-Reynals has shown the influence of the age of the host on the type of lesions produced (5, 6, 7), an influence which is probably most pronounced in the chicken-chicken embryo comparison (5).

The experiments presented here seem to show that there is a difference also in the reactivity of the chorio-allantoic membrane of embryonated eggs of different ages to the Rous sarcoma. Although this difference looks very clear in the diagrams there were great variations in the size of the tumours within each group even, and it seems very difficult to obtain fully uniform results. Karnovsky and coworkers, for instance, obtained 5-gram tumours in 8-day embryos (13). Thus the results seem to depend on several factors. Among them must be reckoned the breed of the eggs, the virulence of the tumour and the method of inoculation. Assuming that the

results with this breed of eggs, this method and a Rous sarcoma of this virulence, are reliable, the age of the embryo has still to be taken into consideration when estimating the growth capacity of the Rous sarcoma in embryonated eggs.

The explanation of the phenomenon may of course quite probably be that, in the case of the chorio-allantois and cells of the Rous sarcoma, the question is just a matter of the blood supply of the membrane. According to Dantschakoff and Gagarin (4), the capillaries develop from the ninth day on. If might, however, equally well be a question of, for instance, the type and amount of nutritional substances present in the chorio-allantois at the time of inoculation, or, probably of suppressing agents present in the earlier phase of development of the embryo. It so, it would be very tempting to make a closer study of the probable factors involved in the activation or suppression of the growth of the Rous sarcoma and other types of tissue in the embryonated chicken egg.

SUMMARY

The size of the Rous sarcoma grown on the chorio-allantoic membrane of embryonated chicken eggs was compared with the age of the embryo at the time of inoculation.

It was shown that the largest tumours were obtained in 11-day embryos, and that the tumour growth in embryos of 7, 8 and 9 days was about two days behind that of embryos 10, 11, 12 and 13 days old. The tumours in the embryos of 7, 8, 9 and 13 days never reached the same mean size as those in the embryos 10, 11 and 12 days old.

The probable cause and implications of these differences were briefly discussed.

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Les objectifs immédiats de ce mouvement sont:

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2) De défendre la valeur du titre d'Ancien Elève Diplômé de l'Institut Pasteur contre les abus ou usurpations, mais aussi de défendre en toutes circonstances les intérêts et prérogatives de ceux qui y ont droit, en particulier dans le domaine administratif et professionnel à l'occasion des concours publics et privés, dans la mesure des Pouvoirs de l'Association en France et à l'Etranger.
3) De procurer aux Anciens Elèves des informations et renseignements sur l'actualité scientifique en Microbiologie et Immunologie, sur les places vacantes et postes à pourvoir, et en général de promouvoir entre tous les bénéficiaires du Titre les services de solidarité qui pourraient être jugés désirables.

Seule représentative, l'Association, constituée selon la loi du 1er Juillet 1901, est placée sous le patronage de la Direction de l'Institut Pasteur, sous la Présidence d'Honneur de Monsieur le Professeur Pasteur Vallery-Radot Membre de l'Académie Française et de l'Académie Nationale de Médecine et de Monsieur le Professeur Jacques Tréfouël, Membre de l'Académie des Sciences et de l'Académie Nationale de Médecine.

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